Vol. 37, No. 4 December 1950

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

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CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 51 MADISON AVENUE

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ON THE BACTERIA RESPONSIBLE FOR SOFT ROT IN STORED POTATOES, AND THE REACTION OF THE TUBER TO INVASION BY *BACTERIUM CAROTOVORUM* (JONES) LEHMANN & NEUMANN

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(With Plate 15 and 3 Text-figures)

Bacterium carotovorum (Jones) Lehmann & Neumann and *Pseudomonas syringae* van Hall were the only two species of Gram-negative bacteria isolated from rotting potatoes collected from clamps in England in 1945–7. Both were found capable of producing a rot under known conditions, and both were isolated on plates of a pectate-gel medium which is liquefied by these bacteria. *Ps. syringae* has not been recorded before as causing a rot of stored potatoes. In a slightly different type of rot which was ropy or gassy and often pink in colour, Gram-positive spore-forming bacilli were found, generally associated with *Bacterium carotovorum*. These bacilli proved to be anaerobes—species of *Clostridium*—one of which, when inoculated together with *Bacterium carotovorum*, produced a gassy rot pink in colour.

On infection by *Bact. carotovorum* the tuber reacts to form a barrier of suberized cells, the extent and efficiency of which depend on temperature and relative humidity. Low temperatures and a low humidity favour the formation of the barrier and eventually of periderm; high temperatures favour the multiplication of the bacteria the advance of which is limited by humidities not reaching saturation. Fluctuations of temperature or humidity may promote a succession of barriers.

The investigation described below formed part of the work on bacterial rots of potato carried out by the junior author from July 1945 to August 1948, in association with W. J. D.

NON-SPORING BACTERIA

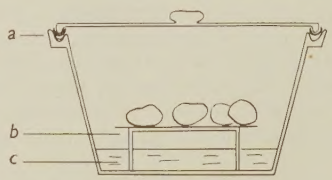
Isolation

Between October 1945 and June 1947 potato tubers showing symptoms of bacterial rot were collected from commercial and experimental clamps, and others were received from Advisory Plant Pathologists throughout the country. After thorough washing the tubers were cut open with a sterilized scalpel, and small pieces of the rotted tissue at the edge of the rot were suspended in sterile water for 20 min. Dilution and streaked plates of the suspensions were prepared using both a meat-infusion agar of pH 7·2 and a pectate-gel medium containing 2% sodium pectate powder in place of the 1% originally devised (Rudd Jones, 1946). Streaked plates were more satisfactory for the latter medium than poured ones, since it was difficult to obtain a good dispersion with the rather viscid pectate-gel.

Colonies of bacteria able to break down pectin appeared after 48 hr. at 25° C. as small craters of liquefied medium, and were readily isolated in a pure state. In order to isolate soft rot bacteria from meat-infusion agar plates carrying a large number of similar colonies, samples of colonies were inoculated as stabs into tubes of the pectate-gel medium. Liquefaction of this medium indicates the splitting of the pectate unit, and comparative tests showed that loss of coherence in plant tissues and liquefaction of the gel were correlated. In other words, an organism that would liquefy the pectate-gel would also dissolve the middle lamella of cells, and vice versa.

Pathogenicity

Isolations which liquefied the pectate-gel were then inoculated into sound potato tubers. The tubers were surface-sterilized by first wetting in methylated spirit followed by immersion in 0·1 % mercuric chloride for 10 min., and finally washed in running tap water for 10 min. Inoculation was done by stabbing to a depth of 2 cm. with a needle charged with the growth from a 24 hr. agar slope culture. The punctures made by the needle were sealed with vaseline and the tubers, supported on a glass sheet, were incubated at 25° C. for 3 days in china chambers containing water and closed with lids sealed with split rubber tubing (Text-fig. 1). This ensured that the atmosphere within the chambers remained near to saturation, which was found to be essential for the establishment of a soft rot.



Text-fig. 1. Incubation chamber for inoculated tubers. *a*, lid with split rubber tubing; *b*, sheet of glass on glass tripod to support tubers; *c*, sterile water.

Twenty-five isolations of soft rot bacteria were made from rotting tubers. Eight were obtained from seed tubers, two of which showed symptoms of blight infection. Seventeen were from ware potatoes of which eight were also blighted; two were infected with dry rot (*Fusarium caeruleum* (Lib.) Sacc.), one showed symptoms of blight and dry rot, one was from a tuber infected with *Pythium ultimum* Trow, and five were only affected with soft rot. Of these twenty-five isolations, twenty-three corresponded to *Bacterium carotovorum* (Jones) Lehmann & Neumann, and the remaining two to *Pseudomonas syringae* van Hall. All produced an extensive soft rot under the above conditions and were subsequently re-isolated.

This is the first record of *Ps. syringae* from naturally infected potatoes, although Dowson (1941) showed that it was capable of producing a rot when inoculated into tubers under laboratory conditions. *Ps. syringae* is clearly more cosmopolitan than

has previously been realized. The rot caused by this pathogen was very similar in appearance to that due to *Bacterium carotovorum*, but was a little firmer and the reaction of the host cells (see below) was more evident. The rotted tissue was always dark brown or black masked by a greenish tinge due to the bacterial growth.

Anaerobic spore-forming bacteria

The association of spore-forming anaerobic bacteria with rotting potatoes was first observed by van Tieghem (1884), and later by Wehmer (1898). McCoy, Fred, Peterson & Hastings (1926) made a detailed study of the acetone-butyl alcohol organisms, one of which had been isolated from a rotting tuber.

During the inspection of clamps a type of rot was encountered which differed from that due to *Bact. carotovorum* or *Pseudomonas syringae* in being either distended with gas or of a ropy consistency and often pink in colour. Stained smears of the rotted tissue showed numerous Gram-positive bacilli often in process of forming spores.

Isolation

By inoculating a small amount of this material into a maize-meal mash to which a few mg. of ascorbic acid had been added just prior to inoculation, incubating for 24–48 hr. at 37° C., and plating the subsequent growth anaerobically on glucose agar plates, several species of *Clostridium* were isolated. These were grown in tubes of meat-infusion broth and ascorbic acid. One of them produced a pink colour in the mash and corresponded closely with *Cl. roseum* McCoy & McClung (1935), which has not hitherto been recorded for Britain. This *Clostridium* produced a gassy rot of raw potato slants which later collapsed into a slimy mass. The starch of both the potato slant and the maize mash was completely hydrolysed and both gelatin and pectate-gel were liquefied. When inoculated into potato tubers in the way already described no rot ensued; but when mixed cultures of *Bacterium carotovorum* and the *Clostridium* were used an active rot developed after 3 days at 25° C. The rotted tissue was distended with gas bubbles and later became ropy. Stained smears of the rotted tissue showed large numbers of spores and both Gram-positive and Gram-negative bacilli. The extent of the rot was considerably greater than that produced by *Bacterium carotovorum* alone under similar conditions. The host tissue never showed any sign of a reaction such as resulted from inoculation with *Bact. carotovorum* (see below), which was to be expected since the semi-anaerobic conditions set up would have prevented the reaction taking place.

The importance of starch-splitting, and, in some instances, pectin-destroying anaerobic spore-formers in storage rots of potatoes has not been generally realized. After infection by various parasites, the host tissues may be stimulated to a more rapid rate of respiration which, coupled with that of the parasites, would result in a rise of temperature and a fall in oxygen tension, conditions favourable for the establishment of anaerobes. Many species of *Clostridium* are known to be active

at temperatures above that of normal storage, and it is suggested that there is a sufficient rise in temperature of tubers, following infection by *Bacterium carotovorum*, to allow of the initial growth of these anaerobes, which, in turn, may lead to a further rise in temperature. Once infection has taken place, the various butyric acid organisms (*Clostridium* spp.) are able to grow and multiply, since there will be an abundant supply of starch in rotting tubers. The ability of these bacteria to hydrolyse starch enables them to compete with, and suppress, other bacteria, which would explain the almost pure cultures of these anaerobes sometimes found in the later stages of bacterial rots of stored potatoes.

The reaction of the potato tuber to invasion by *Bacterium carotovorum*

The formation of periderm by the potato tuber as a response to infection by pathogenic fungi and bacteria has been observed by Appel (1906), Leach (1931) and others. Priestley & Woffenden (1922) stated that suberization of cell walls precedes the formation of periderm as a reaction to wounding; Herklots (1924) showed that a pH of 6·4 was optimal, and that at pH 7·5 and above, wound cork was not formed although suberization was active. Artschwager (1927) found that both temperature and humidity affected suberization. The following experiments were designed to investigate the effect of altering these two conditions in one and the same tuber.

Tubers of King Edward, Majestic and Arran Banner were surface-sterilized in the way already described, and cylinders of tissue were removed from each with a flamed cork borer, 2 mm. in diameter, provided with a collar so that the plug of tissue removed was exactly 2 cm. in length. The inoculum, a 24 hr. old meat-infusion broth culture of *Bact. carotovorum*, was introduced by a hypodermic syringe. When filled the cavities were closed with small glass plugs previously sterilized by heat. The inoculated tubers were incubated in the china chambers already described for 3 days at temperatures of 5, 10, 15, 20 and 25° C. Controls with sterile water in place of the bacterial culture were maintained under similar conditions. In one series the relative humidity was lowered to 86% by replacing the water in the chambers with 23·5% sulphuric acid.

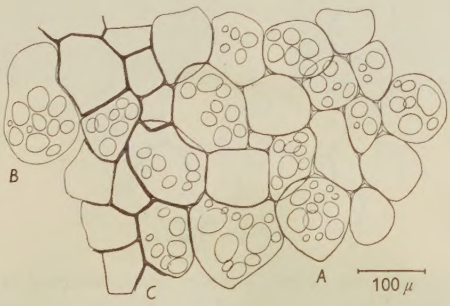
At the completion of the treatment the tubers were cut open and samples of tissue at the advancing edge of the rot were fixed in Belling's modification of Navashin's solution or in formol-acetic alcohol for embedding in paraffin wax. Microtome sections, 18–24 μ thick, were cut and stained with either thionin and orange G (Stoughton, 1930), Sudan IV, or ammoniacal gentian violet. Freehand sections were also cut and mounted unstained in water.

The host reaction is a thickening of the walls at the advancing edge of the rot (Text-fig. 2). Seen unstained in water these suberized cells were dark brown; they were deeply stained with Sudan IV, ammoniacal gentian violet, and thionin. The thionin-orange G combination gave the best results, since the cell walls of

healthy tissue took up the orange G, while the thickened cell walls were stained with the thionin. The bacteria in the intercellular spaces were also stained with the thionin which was also slightly retained by the walls of completely separated cells. The thickening imparted a certain hardness to the rotted tissue and such suberized cells were not readily separable.

At temperatures above 20° C. and in a saturated atmosphere for 3 days, the bacteria had spread so rapidly that the host cells were killed before suberization commenced and no browning of the tissue was apparent (Pl. 15, fig. 2).

At a temperature of 20° C. and in a saturated atmosphere for 4 days, following 3 days' incubation at 25° C., the subsequent suberization was very slight in King Edward and not visible in Majestic. The rotted tissue was slightly discoloured in the former; but histological examination revealed no appreciable thickening of the cell walls.



Text-fig. 2. Effect of temperature on host reaction. Freehand section of Arran Banner tuber, showing one rotted cell (*B*) lying free and separated from the healthy tissue (*A*) by a barrier (*C*). Camera lucida drawing.

Temperatures of 10 and 15° C. for 3 days, following 3 days' incubation at 25° C., produced marked reactions. In King Edward at 15° C. the rotted tissue was brown and the cell walls were slightly thickened; at 10° C. the effect was accentuated. The cells had reacted to the bacteria, but the thickening was not sufficient to stay the invasion though its progress was retarded. A number of cell layers were suberized. Thus, at 15° C. 12–15 layers were affected, at 10° C. only 5–7; but the thickening was greater and the retarding effect had increased. At 5° C. the reaction was even more pronounced, the thickening had increased and fewer cells were involved, 2–3 layers in King Edward, 1–3 in Arran Banner (Text-fig. 3).

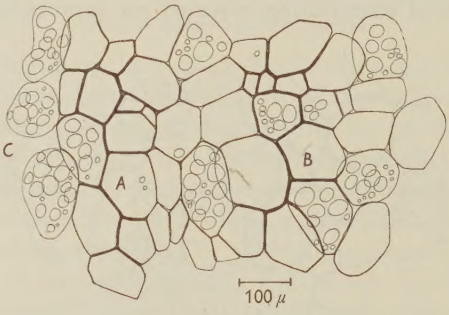
Under these conditions (5° C. in a saturated atmosphere following incubation for 3 days at 25° C.) suberization had prevented the further advance of the bacteria in King Edward and in Arran Banner but not in Majestic.

When inoculated tubers were again subjected to a temperature of 25° C., following 3 days of 5 or 10° C., the bacteria penetrated the barrier and produced a further rot, provided that cork formation had not commenced (uninoculated tubers showed

no reaction at these temperatures). Tissue behind the barrier reacted slightly when incubated at 25° C. for the second time, although uninoculated tubers did not react at this temperature. A second barrier was formed when the tubers were again subjected to a lower temperature (Text-fig. 3 and Pl. 15, fig. 3).

Effect of lowering humidity

Lowering the humidity to 86% had a similar effect as lowering the temperature (Pl. 15, fig. 4), i.e. suberization increased. This effect increases as the tissues dry out and the reaction at the lowered humidity eventually stops the advance of the bacteria by the formation of a cork barrier.



Text-fig. 3. Freehand section of Arran Banner tuber, infected with *Bact. carotovorum*, showing two host reaction barriers, (*A*) and (*B*), produced by variations in the incubation temperature. Rotted cells at (*C*). Camera lucida drawing.

It may be concluded, therefore, that the efficiency of a barrier of suberized cells depends upon temperature and humidity. Low temperatures (5–10° C.) and a low relative humidity (86%) favour the formation of a retarding barrier, while, on the other hand, high temperatures (20–25° C.) favour the multiplication of the bacteria, which will spread provided the humidity is sufficiently high. Fluctuations of temperature or humidity alternately favour the host reaction or the spread of the bacteria, and consequently a series of host reaction barriers may be produced.

The junior author is most grateful to the Agricultural Research Council for a grant enabling him to carry out this investigation. Both authors wish to thank Prof. F. T. Brooks for his interest in the work.

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Fig. 1

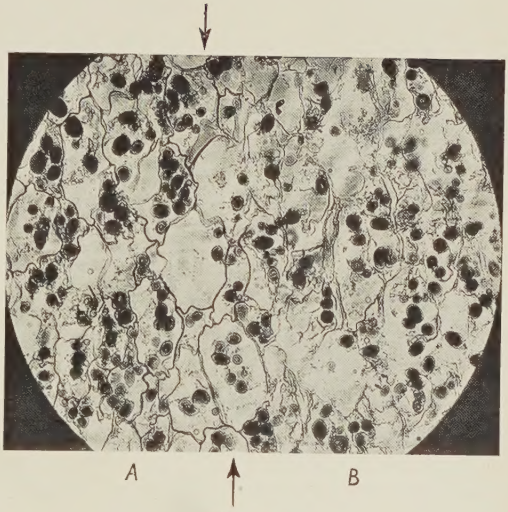

Fig. 2

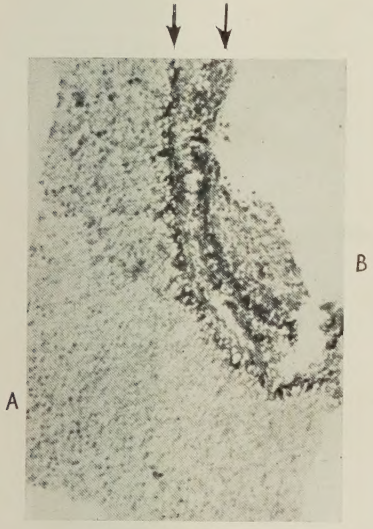

Fig. 3

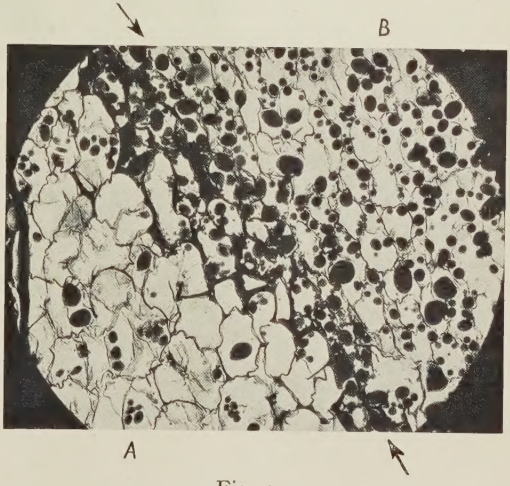

Fig. 4

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EXPLANATION OF PLATE 15

Fig. 1. Left: Majestic tubers naturally infected with soft rot bacteria. Right: same tubers in section. The advance of the bacteria has been stopped by a layer of barrier cells.

Fig. 2. Section of King Edward tuber infected with *Bact. carotovorum*, the arrows showing the boundary of the healthy tissue (*A*) and the rotted tissue (*B*)—no reaction of host cells. × 58.

Fig. 3. Section of King Edward tuber infected with *Bact. carotovorum*, showing two host reaction barriers, indicated by arrows, produced in relation to variations in the incubation temperature. Healthy tissue (*A*), rotted tissue (*B*). × 12.

Fig. 4. Effect of humidity on the host reaction. Section of King Edward tuber showing healthy (*A*) and rotted (*B*) tissue, separated by a barrier of suberized cells indicated by arrows. × 58. See Text-fig. 2.

(*Received* 10 *February* 1950)

WHEAT POWDERY MILDEW INVESTIGATIONS

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The development of *Erysiphe graminis* f. *tritici* Em. Marchal, from spore germination to spore formation, occupied 6 days and ascospore discharge in the field covered a period of 1 month. On immune hosts development of the fungus generally ceased at the point of invasion of cell cytoplasm. The features of host plant resistance have been discussed.

Lithium chloride soil applications were made as 0·8% solution in glasshouse seedling plant experiments and at 56 lb./acre rate in a factorial field trial. In the former, mildew was reduced from 11% leaf area infected to 1%, but under field conditions only from 1·29 to 1·04% over two sampling periods. The limiting effects of lithium on mildew began to decline 18 weeks after soil treatment.

Race 4 of *E. graminis* f. *tritici* (Canadian typing) was present in all, and Race 3 in about half the New Zealand wheat localities during 1948–9. The infection types of Races 3 and 4 on 180 wheat varieties and breeding selections have been recorded, and the basis for a mildew resistance plant-breeding programme is discussed, including consideration of adult plant resistance.

Morphology, host infection, resistance

Methods

Infected wheat leaves for microscopic examination were decolorized by boiling in 70% alcohol and stained with cotton-blue in lacto-phenol. In studying fungus penetration, satisfactory free-hand sections of infected leaves were prepared using chromoacetic fixative, followed by sectioning in pith and mounting in warm blue lacto-phenol. Several other procedures involving dehydration, mordanting and staining were attempted, but with several transfers the hyphae became detached and the simpler procedure alone was satisfactory.

Development of Erysiphe graminis *f.* tritici

The development of colonies on Cross 7 wheat leaves was studied by inoculation with conidia followed by daily examination. The germinating spore usually formed one germ tube which became septate and swollen near the apex. From the apical region an infection process arose, penetrated the leaf cuticle and gave rise to a subcuticular haustorium. The mycelium then grew rapidly, and the tendency of hyphae to follow hollows between the long narrow epidermal cells probably accounted for the elongate nature of the fungus colony in early stages.

Secondary haustoria numbering from 1 to 8 (with a mean of 5) were found in all colonies on the 4th day. Small swellings on the surface of the central hyphae appeared

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after 5 days, giving rise to conidiophores which were fully developed at 6 days, at which stage the fungus was just visible to the eye.

Perithecia were formed on infected plants at all ages during the summer months, and the mycelial mats at this stage were uniformly covered with curved colourless hairs. Under field conditions ascospore discharge continued from a month in early autumn and infection of volunteer wheat plants was observed shortly after, in April (late autumn).

Conidia were germinated within a wide range of 6–34° C. when humidities varied from 40 to 100% on both susceptible and resistant hosts. Conidial germination did not appear to be a critical phenomenon associated with host infection. Penetration of cell walls was observed in *Triticum vulgare*, *T. monococcum*, *T. timopheevi*, *T. persicum*, *Bromus unioloides*, *Hordeum murinum*, *H. distichon*, *Sonchus arvensis*, *Capsella bursa-pastoris*, *Agropyron scabrum*, but no haustoria were found in many of these.

Halos in the stained preparations caused by enzyme action on the cellulose tissue occurred round the penetration points. Corner (1935) had observed that on resistant material infection proceeded as far as the formation of infection papilla, though sometimes haustoria and conidiophores were formed. In the work under consideration, on most resistant hosts and in all cases on immune hosts, the development of the fungus was stopped at the point of cell cytoplasm invasion. On some resistant hosts haustoria were formed in invaded epidermal cells, but these failed to stain with aniline blue and soon became disorganized as described by Salmon (1904).

It appeared that severe infection of wheat with *Erysiphe graminis* f. *tritici* depended on the protoplasmic reaction of the host plant cells as determined by a particular genotype, and modified by certain environmental conditions. The reaction of chlorenchyma tissue underlying invaded epidermal cells appeared also to play an important part in resistance in some varieties which showed large necrotic lesions. It was thought this necrosis could be caused either by toxins liberated by the fungus within cells, or by a rapid loss of nutrients withdrawn by the parasite.

Infection types

The infection types proposed by Mains & Dietz (1930) for *E. graminis* on barley were used for recording reactions:

Type 0. Highly resistant; slight flecking or necrotic spots.
Type 1. Very resistant; slight mycelium, very slight spore production.
Type 2. Moderately developed mycelium, slight spore production.
Type 3. Moderately susceptible; moderately developed mycelium and sporulation.
Type 4. Very susceptible; abundant mycelium and sporulation.

The necrotic host reaction seemed to be of particular interest and was most marked among *Triticum durum* varieties in which the affected areas consisted of

both epidermal and mesophyll cells. A milder degree of necrosis occurred on *T. vulgare* leaves derived from Hope and H. 44 crosses, varying from a faint yellowing to a light brown flecking of inoculated leaves. In the wheat variety resistance trials (below) the F_1 Hope and Pilot crosses showed Type 2 or 3 infections, but only fourteen of the 180 varieties examined in the main trial. The bulk of these were in two main groups, Types 0–1 and Type 4.

Resistance

The basis of host resistance to powdery mildew is generally recognized as being primarily dependent on the protoplasmic reaction of the plant cells, but there is some evidence of the effects of morphological features. Thus Germar (1935) showed that increased resistance occurred in plants with silicic acid supplements and considered that the effect was associated with a higher silica content of cell membranes. In the material he studied Homma (1937) concluded that morphological characters such as fewer surface hairs and fewer thick-walled epidermal cells were associated with susceptible wheat varieties. In the wide range of resistant and susceptible varieties tested in the work now being described, no evidence was obtained of any correlation of hairiness or cell-wall thickness with host resistance. Chester's (1946) analysis of the problem of physiologic specialization in the leaf rusts seems to fit the observations made on *Erysiphe graminis*, namely, that in a wide view, the hosts susceptible to obligate parasites are in a minority. These susceptible hosts probably possess some chemical compound which is necessary for development of infection, and because of the wide range of races of the parasites this compound is more probably a specific protein than a carbohydrate or inorganic compound. One may postulate the action of a protein as being either a food base used by the fungus, or a protein antitoxin counteracting toxins liberated by the fungus. In his studies with wheat and barley, Cherewick (1944) concluded that the more compatible the host is with the pathogen the slower is the disintegration of host cells and haustoria and the more severe is the infection. Such a conclusion would fit the hypothesis of specific proteins required by the fungus, since the lack of these in resistant hosts would cause the death of host cells by toxins liberated by the dying fungus hyphae. Sempio (1939) postulated two forms of protoplasmic resistance of wheat to *E. graminis*: first, toxicological resistance which results from the presence of regression products or absorbed toxins; secondly, metabolic resistance by means of which the soluble components of protoplasm are rendered less attractive to the parasite which becomes weakened through lack of food. Chester's hypothesis of specific proteins varying in quantity with different environmental conditions conforms to metabolic resistance, while the evidence of experiments with applications of lithium chloride (Table 1) affords some evidence of toxicological resistance.

Manurial experiments

In preliminary glasshouse pot experiments, four wheat varieties in soil and sand respectively were used, with comparisons between the effects on mildew infection of lithium chloride 0·8% solution and a complete mineral nutrient solution. Half the plants had been inoculated at emergence and the remainder, protected by 'Cellophane' covers, were inoculated after 3 weeks' growth. Over the whole experiment lithium chloride reduced mildew infection from 11% (leaf area) to 1%, and the leaf length of plants was reduced from 167 to 137 mm., representing a decrease in growth of one-sixth but an almost complete elimination of mildew.

Kent (1941) has reviewed other experimental work relating to effects of lithium salts in reducing powdery mildew, and from his experiments was able to deduce that at about 30 mg. lithium/100 g. of growing fresh material, the mildew infection would approximate to zero.

In mycological observations associated with the current investigation, malformed globose haustoria were observed on infected leaves of lithium-treated plants, together with abnormally swollen hyphae and twisted conidial chains. When conidia germinated on lithiated plants, fungus development stopped in all cases after penetration of the cell wall.

Experiments, as described by Kent (1941), were conducted on glasshouse-grown wheat plants sampled at an early stage of growth. With the results obtained on young plants in mind it was considered desirable to undertake a factorial field plot experiment as follows.

Experimental

The effects of lithium chloride, superphosphate and sulphate of ammonia were compared on the autumn-sown varieties, Cross 7, Fife-Tuscan, Dreadnought, Tainui, WRI-Yielder and Hilgendorf. There were two sowing rates of twenty-four and one seed per foot of the rows which were 14 ft. long in triplicate. The fertilizer treatments were:

(1) nitrogen (224 lb. sulphate of ammonia per acre),
(2) phosphorus (448 lb. superphosphate per acre),
(3) lithium (56 lb. lithium chloride per acre),

and the following combinations at the same rates as above:

(4) nitrogen + phosphorus + lithium,
(5) nitrogen + phosphorus,
(6) nitrogen + lithium,
(7) phosphorus + lithium,
(8) nil.

The nitrogen and phosphorus fertilizers were applied immediately before seed sowing and the lithium as a water solution applied before plants were inoculated.

Two guard rows of Cross 7 wheat were sown through the centre and round the edge of the whole trial block. The central guard rows of Cross 7 were dusted 6 weeks after sowing with a mixture of conidia and talc, and severely infected wheat seedlings were transplanted into the two central guard rows. After 10 days, mildew infection was noticed on the central guard rows and the adjoining plots. Leaf samples for mildew determinations were taken from the middle row of each plot using ten green leaves sampled 6 weeks after inoculation of the guard rows. A second recording using fifteen leaves was made after 3 months. The results are represented in Tables 1–3.

Table 1. *Field factorial experiment. Means and significant differences for (a) blocks and sowings and (b) varieties and manures*

(*a*) Blocks and sowings

			Difference for significance	
	North	South	1%	5%
Blocks:				
June, mildew (%)	0·266	2·065	1·59	1·27
June, leaf length	15·354	14·729	6·70	5·36
August, mildew (%)	0·700	1·382	0·51	0·41
August, leaf length	17·940	18·330	0·53	0·42
	I, spaced 1 ft.	II, normal		
Sowings:				
June, mildew (%)	0·98	1·35	1·54	1·23
June, leaf length	14·77	15·31	1·39	1·11
August, mildew (%)	0·32	1·73	0·35	0·28
August, leaf length	17·46	18·58	0·65	0·52

(*b*) Varieties and manures

	Cross 7	Fife-Tuscan	Hilgen-dorf	WRI-Yielder	Tainui	Dread-nought	Difference for significance 1%	5%
Varieties:								
June, mildew (%)	0·83	0·58	2·28	1·56	1·24	0·51	2·75	2·2
June, leaf length	14·94	16·25	14·16	15·63	15·47	13·81	11·62	9·30
August, mildew (%)	1·31	0·23	1·11	1·96	1·44	0·10	0·89	0·71
August, leaf length	17·75	17·88	17·63	18·19	19·00	18·38	0·92	0·74

	Sulphate of ammonia		Superphosphate		Lithium chloride			
	N	Nil	P	Nil	Li	Nil		
Manures:								
June, mildew (%)	1·19	1·14	1·36	0·97	1·04	1·29	0·23	0·19
June, leaf length	15·08	15·00	15·49	14·59	14·96	15·13	1·71	1·37
August, mildew (%)	1·69	0·36	1·17	0·89	0·83	1·22	0·56	0·45
August, leaf length	18·95	17·31	18·56	17·71	17·96	18·31	0·58	0·46

Discussion of results

The results of leaf length showed no differences in either June (winter) or August (spring) between north and south blocks. However, in both June and August there was an increased mildew percentage in the south compared with the north block, a variation apparently resulting from spore distribution by prevailing winds. In the period May–June–July there were 46 days with northerly winds and 24 with southerly. The difference between the two blocks was smaller in August, but the observation indicates that the wind is a very important agent in mildew distribution.

TABLE 2. *Field factorial experiment. Means and significant differences. Mildew percentage (spring 1949)*

Treatment	Cross 7	Fife-Tuscan	Hilgendorf	WRI-Yielder	Tainui	Dreadnought	Difference for significance 1%	5%
South+spaced	0·68	0·43	0·33	0·79	0·38	0·10	1·77	1·42
South+normal	3·08	0·40	2·76	4·32	2·86	0·03	1·77	1·42
North+spaced	0	0·06	0·34	0·16	0·13	0·16	1·77	1·42
North+normal	1·49	0·03	0·99	2·55	2·11	0·10	1·77	1·42
South (block)	1·88	0·42	1·55	2·56	1·62	0·65	1·25	1·00
North (block)	0·74	0·05	0·67	1·36	1·24	0·13	1·25	1·00
Spaced (sowing)	0·34	0·25	0·34	0·48	0·38	0·13	0·85	0·68
Normal (sowing)	2·28	0·22	1·88	3·44	2·49	0·06	0·85	0·68
N (nitrogen)	3·06	0·06	2·60	2·61	1·83	0·06	2·72	2·18
P (phosphate)	0·80	0	0·46	0·19	0·66	0	2·72	2·18
Li (lithium)	0·73	0·06	0·53	0·49	0·49	0·13	2·72	2·18
NPLi	2·93	0·06	2·60	2·13	2·00	0·06	2·72	2·18
NP	2·13	1·0	1·56	6·33	3·23	0·20	2·72	2·18
NLi	0·73	0·46	0·59	2·19	1·86	0·19	2·72	2·18
PLi	0	0·06	0·26	0·66	0·59	0·13	2·72	2·18
0 (Nil)	0·13	0·13	0·26	1·06	0·80	0	2·72	2·18
	1·31	0·23	1·10	1·96	1·43	0·10	0·95	0·71

Treatment	N	P	Li	NPLi	NP	NLi	PLi	0	Difference for significance 1%	5%
Spaced	0·36	0·11	0·23	0·44	0·57	0·26	0·17	0·39	1·57	1·26
Normal	3·04	0·59	0·58	2·82	4·24	1·75	0·39	0·39	1·57	1·26
	1·70	0·35	0·40	1·63	2·41	1·00	0·28	0·39	1·11	0·89

The mildew incidence in August with normal sowing was nearly six times as severe as with widely spaced plants, although the leaf length was increased by only 6·4%. No varietal differences occurred in June in leaf length or mildew percentage. At the spring sampling, on the other hand, Dreadnought and Fife-Tuscan were more resistant to mildew than Cross 7, Hilgendorf, WRI-Yielder and Tainui. No differences were found between Cross 7, Hilgendorf and Tainui, but WRI-Yielder was more susceptible than Hilgendorf. The same order of resistance occurred after 4 months'

growth in this trial as had been found in a wheat-district survey of crops examined after 9 months' growth. This fact emphasizes the importance of so-called 'adult plant resistance' which appears to be important in the field during the main growing period.

Of the fertilizer applications, superphosphate gave a very marked growth increase in June, with which increased mildew was also associated. Nitrogen applications in June produced only a slight growth increase and no mildew increase. Lithium chloride at this winter period caused a slight growth reduction and a large reduction

Table 3. *Analysis of variance. Mildew percentage infection and leaf length of wheat varieties in winter and spring*

				June				August			
		'F.' value at		Mildew (%)		Leaf length		Mildew (%)		Leaf length	
	D.F.	1% level	5% level	Mean sq.	'F.' value	Mean sq.	'F.' value	Mean sq.	'F.' value	Mean sq.	'F.' value
Whole plots:											
Blocks B	1	16·26	6·61	77·58	8·0*	93·75	>1	10·094	10*	3·82	2·05
Varieties V	5	10·97	5·05	7·29	>1	137·23	>1	8·384	8*	4·12	3·79
Error B×V	5	—	—	9·72	—	172·93	—	1·009	—	—	—
Total	11	—	—	—	—	—	—	—	—	—	—
Half plots:											
Sowings S	1	16·26	6·61	3·19	3·5	70·42	9·4*	47·700	94†	25·01	15·4*
S×V	5	10·97	5·05	1·04	1·1	32·21	4·3	5·963	12†	3·96	2·44
S×B	1	16·26	6·61	4·82	5·3	60·00	8·0*	3·507	7*	3·76	2·32
Error S×V×B	5	—	—	0·91	—	7·45	—	0·472	—	—	—
Total	23	—	—	—	—	—	—	—	—	—	—
Sub-Plots:											
Manures M	6	3·47	2·42	1·24	3·1*	44·43	4·1†	9·043	7·5†	15·52	12·0†
N	1	7·56	4·17	0·05	0·2	1·67	>1	42·307	35·2†	65·01	50·0†
P	1	7·56	4·17	3·72	18·5†	192·60	16·5†	2·027	1·7	17·51	13·4†
Li	1	7·56	4·17	1·38	6·8*	6·67	>1	3·531	2·9	3·01	2·3
NP	1	7·56	4·17	2·071	10·35†	17·61	1·5	3·341	2·7	3·76	2·9
NLi	1	7·56	4·17	0·019	0·1	10·41	>1	3·014	2·5	2·34	1·8
PLi	1	7·56	4·17	0·03	0·15	37·61	3·3	0·038	>1	0·26	>1
S×M	6	3·47	2·42	0·4	2·0	8·47	>1	6·490	5·4†	2·95	2·3
V×M	30	2·38	1·84	0·64	3·2†	14·31	1·2	1·168	>1	1·36	1
Error V×S×M	30	—	—	0·206	—	11·37	—	1·208	—	1·30	—
Total	95	—	—	—	—	—	—	—	—	—	—

* Significant at 5% level.
† Significant at 1% level.

in mildew infection. In the spring (August) the effect of phosphate on growth was reduced and there was no increased mildew percentage, but at this stage sulphate of ammonia was causing much greater growth increase and mildew percentage infection. At this spring period the lithium application continued to show plant growth and mildew reduction, but the differences in mildew were not significant over the whole experiment. The reduction in mildew in the field on older plants

was certainly much less noteworthy than in the glasshouse trial, and there was evidence that the effects in the field were beginning to disappear 18 weeks after lithium application.

Physiological specialization and varietal resistance

Experimental

Though many varieties of the genera *Triticum*, *Hordeum*, *Avena* and *Agropyron* were grown during 1948–9, infection by *Erysiphe graminis* f. *tritici* occurred in the field only on the susceptible species of *Triticum vulgare*, *T. polonicum* and *T. compactum*. Over the New Zealand wheat-growing area infected (*T. vulgare*) plants were collected in widely separated localities, transferred to the glasshouse and infections re-established by transfer back to susceptible Cross 7 variety seedlings. Conidia from these were inoculated on to the differential varieties, Axminster R.L. 75, Chul R.L. 543, Huron R.L. 20, and Norka R.L. 1888, used by Cherewick (1944) who kindly supplied the seed. The New Zealand races were identified following the procedure outlined by Newton & Cherewick (1947) in Canada. Inoculations were made by touching two pustules of mildew against each leaf of all varieties tested and such inoculations were made just before second leaves appeared. Before and after inoculation the seedlings were placed with non-inoculated susceptible control seedlings under cellophane covers. In all cases no stray infection was present on the susceptible control plants when the differentials were scored.

In 1948, Race 4 was found in all the wheat districts, while Race 3 occurred in half of them. In 1949, additional tests were conducted with plants inoculated by means of moistened perithecia from samples originating throughout the growing region. Races 3 and 4 only were recorded, with the latter predominant.

Isolation of Erysiphe graminis *f.* tritici

Races 4 and 3 were obtained by testing single mildew pustules obtained after transferring infection (Race 4) from Cross 7 to susceptible Axminster, then to Huron and finally back to Cross 7, or with Race 3 from Cross 7 to Huron, to Chul, and back to Cross 7. These transfers were all made with single pustules in an attempt to isolate single races and after the final transfer three single pustules were inoculated on to the differential varieties (two replicates each). The results are shown in Table 4.

Table 4. *Infection types on wheat differentials by six mildew isolates*

Isolate	Axminster	Chul	Huron	Norka
Race 4*a*	0, 0	0, 0	4, 0	0, 0
4*b*	0, 0	4, 0	4, 4	0, 0
4*c*	0, 0	0, 0	4, 4	0, 0
Race 3*a*	0, 0	0, –	4, 0	0, 0
3*b*	4, 0	0, 4	4, –	0, 0
3*c*	0, 0	4, 4	4, 4	0, 0

From these results it was observed that isolate 4*b* contained Race 3, but the other isolates were retained under cover as pure Race 4 infection for inoculation purposes and Race 3 was maintained similarly. The isolation of mildew races was also attempted by transferring single spores on to marked areas on wheat seedling leaves. Over forty spores from four separate isolates were transferred but no infection occurred. Possibly better results would have been secured had the spores been first shaken on to sucrose gelatine for 12 hr. Then those which had germinated could have been placed on to selected leaves.

As may be seen from Table 4, one Axminster plant in the 3*b* inoculation became severely infected. On other occasions plants of Norka variety also had become infected. On the chance that races other than 3 and 4 were present, these infections were again tested as single pustules on six separate occasions on the differentials. In each case they reacted as Race 4. Furthermore in the field, susceptible and resistant Axminster plants were found together, from which seed was harvested and seedlings subsequently inoculated with Race 4. The results showed 100% resistance for seedlings from originally resistant plants and 57% resistance for seedlings from susceptible plants. Thus the problem of seed not true to differential type requires care in selecting or harvesting seed for race identification.

Resistance of varieties

Experimental

Field records of the Wheat Research Institute on mildew incidence were used as a basis for selecting the varieties and selections considered worthy of testing against the isolated races. All seedling tests in pots were made under as uniform conditions as possible in a glasshouse with temperatures at all times between 15 and 20° C. Inoculations were made when the second leaf had emerged, by shaking infected Cross 7 seedling plants over those under test and the two race inoculations were made in separate compartments. From ten to forty seedlings of each variety were inoculated, and the recording of infection type on the Mains & Dietz Scale was made on at least two separate occasions between the 10th and 20th day after inoculation. A check on the accuracy of the procedure was made by testing the same group of sixty-five varieties on three separate occasions. In only one variety was there a difference of three grades in infection type recorded, while a difference of two grades was found in only three varieties. The results showed that one test was accurate to within one infection type in 93% of the varietal material under examination.

Results

The infection types of *E. graminis* f. *tritici* Races 3 and 4 have been recorded on 180 wheat varieties and selections at present included in the breeding programmes of the New Zealand Wheat Research Institute. The results show that within the species *Triticum vulgare* there were fifty-six varieties completely resistant to both races, and

it would appear that there is abundant breeding material for the development of varieties resistant to the dominant races of the fungus. These fifty-six varieties included the following:

(1) Varieties with H. 44–24 parentage, e.g. Merit, Regent, Renown; with Hope parentage, e.g. Hofed 1, Pilot, Warigo, D. 15.

(2) Fourteen varieties from India or with one parent of Indian origin.

(3) Kenya S. 2250 and S. 2386 and D. 10 (one Kenya parent).

(4) Eight Pentad crosses including Coronation.

(5) The remaining varieties possessing complete resistance to both races were: April Bearded, Axminster, Minred A, Norka, Parker's Selection, Red Bobs, Redman, Saunders, Tajir S. 2438, S. 2449, Vermilion and 28801.

Varieties resistant to Race 4 and susceptible to Race 3 were: Afghanistan S. 2177 and S. 2188, Bomen, Chul, India S. 1898, India 38, Tajir S. 2441.

Six varieties susceptible to Race 4 and intermediate (Type 2 or 3) in reaction to Race 3 were: Caleb, Kanred, Lin Calel, Malakof, Marquis × Kanred S. 1687, Marquis × Kanred S. 1688.

The one variety resistant to Race 4 and intermediate in reaction to Race 3 was Kenya S. 2250. Carrabin was intermediate in reaction to Race 4 and susceptible to Race 3.

Only two varieties showed intermediate (Type 2) resistance to both Races 3 and 4. They both possessed 'Hope' resistance, since they were F_1 progeny of Hope × Cross 7 and Pilot × Cross 7 which indicated that the inheritance of Hope resistance was recessive, as shown originally by Mains (1934).

In other instances where varieties and races corresponded, confirmation was obtained in New Zealand of the variety reactions recorded in Canada by Newton & Cherewick (1947).

Adult plant resistance

When the comparison of seedling and field-infection types was made, it was noted that many varieties susceptible in the early stage were resistant as adult plants. The value of adult plant resistance depends of course upon whether it is effective when disease intensity is at the maximum. Evidence was obtained in the field-factorial trial (Tables 1–3) that in the New Zealand standard varieties Fife-Tuscan and Dreadnought, adult plant resistance is present from about 3 months after wheat is sown, which means that it is effective for the period of greatest infection from early spring. Accurate yield trials are still required to determine the effect of mildew on yield, but it appears that adult plant resistance as in Fife-Tuscan and Dreadnought may be sufficient protection against powdery mildew and with an accurate method of recording this form of resistance in the field it may be a relatively simple project to transfer this to the more susceptible varieties. In the plant material examined, adult resistance seemed to occur in the varieties already named, and in Eureka, Holdfast, Reward, White Fife, Yeoman, D. 3, with partial resistance in Cross 7, Fedweb, Jumbuck, Marquis and Thatcher.

Possibly the same sequence may occur with powdery mildew, as Popp (1933) observed in the adult plant resistance of Hope, H. 44–24 and crosses to stem rust. He found that the leaf resistance increased with age and with a gradual increase from the first to last formed leaves. Newly appearing leaves were susceptible for 2 days and then became resistant, and he noted that at ear emergence all the leaves above the fourth were immune.

Inheritance of adult resistance

This was observed by recording the incidence of wheat mildew in both the parents and families of crosses. The families were placed in four groups according to a 'mildew index' obtained by finding the product of the mean infection types of the parents used in the particular family. The mean mildew infection for all the families in each group was then obtained from field recordings of mildew resistance, and the results are shown in Table 5.

Table 5. *The inheritance of adult plant resistance—a comparison of parent and progeny reaction to* Erysiphe graminis *f.* tritici

Group	Parent mildew potential	Family (progeny) mean mildew incidence
A	0	1·8
B	2–4	2·4
C	5	2·4
D	6–12	3·0

These results indicated a similar gradient in mildew susceptibility between parent and progeny, from which it would seem that adult plant resistance in the New Zealand breeding material is strongly inherited.

In Canada, Newton & Cherewick (1947) recorded adult resistance in the varieties Gaza, Kanred, Mindum and Pentad, and they stated that varieties of cereals possessing the adult plant type of resistance might be expected to have considerable value as breeding material, but they considered that a better starting-point for a breeding programme would be to choose as parental material, varieties immune or highly resistant as seedlings.

The work of Swenson (1944) is of significance in this context. He had controlled mildew in spring wheat from flowering time to harvest by applications of fungicidal dusts and over two seasons he found a 3% yield reduction. When dusted throughout the crop-growing season, however, he found from his controls that mildew had caused a 15% yield loss, indicating that the main mildew effect on yield is caused before the crop-flowering stage. The same observation has been made in the New Zealand experiments where sulphur dusts and sprays after flowering of the plants failed to establish a reduction in yield through mildew infection. Therefore adult plant resistance to be of value would have to be present long before flowering time.

The problem of mildew control

Fertilizers and fungicides

A general conclusion from field trials, including the factorial experiment described earlier, is that if there is a marked growth response from any fertilizer there will probably be an increase in mildew susceptibility for the period of growth response. The evidence from normal sown and widely spaced plants indicates that this was due to an effect on humidity within the crop. Within wheat fields sown after grass, 'nitrogen spots' caused by animal droppings are commonly observed, and mildew is to be found among the plants on these spots, even though little occurs elsewhere. It seems that the disease tends to increase when the balance of nutrients is high towards nitrogen and phosphate and also when the total nutrient supply is high, though Stuch (1926) and Trelease & Trelease (1928) have shown that reduced mildew severity may accompany the application of potassium if the general nitrogen-phosphate level is high.

The limiting factor in the use of fungicides is an economic one, even though sulphur fungicides undoubtedly are very effective if they can be applied at the correct time. Their use, however, continues to be impossible in standard wheat practice.

Chemicals which may function systemically, e.g. silicon and lithium, have been found much less effective in field than in glasshouse experiments, while their action seems to be of short duration and the cost of application uneconomic.

Field dissemination

Much could be achieved if there were practicable means of destroying volunteer plants, wheat stubble and straw in late summer. In observations of the seasonal cycle of the fungus in New Zealand it was found that by the end of February (late summer) all wheat powdery mildew infections (conidia) of the previous crops had died out. No infected volunteer plants were found at that time of the year. Perithecia in stubble began shedding ascospores in the last week of February and this liberation of infective inoculum ceased about one month later. On the other hand, perithecia on leaves and straws in stacks were all found to be unopened in April, but their viability was demonstrated after they had been moistened for a few days. This combination of circumstances results in the appearance of volunteer sown and infected plants in the autumn, and it is unfortunate that wheat-farm practice is so organized that no attention can be given either to burial or burning of infected straw and stubble before ascospore liberation.

Plant-breeding possibilities

The wide range of mildew-resistant varietal material may be grouped thus:

(1) Triticum vulgare *varieties with adult resistance*. These include Fife-Tuscan, Cross 7 (partial adult resistance) and progeny of Dreadnought.

(2) T. vulgare *varieties with seedling resistance to at least two Races*. This group includes (for New Zealand) Axminster, April Bearded, Minred A, Norka, Parker's Selection, Red Bobs, Kenya, D. 10, several varieties from India, Coronation, and several Pentad crosses. (*Note:* Axminster and Norka are susceptible to Race 5 in Canada; Red Bobs and Kenya S. 2396 are susceptible to Races 1 and 2 in U.S.A.)

(3) T. vulgare *varieties with mildew resistance derived from* T. durum. These include progeny of Hope and H. 44-24. (*Note:* susceptible to two Races in U.S.A.—see Taylor, Rodenhiser & Bayles (1949).)

(4) *Varieties with mildew resistance from* T. timopheevi, T. monococcum, T. persicum *and rye* (Secale cereale). See Kostoff (1938), Shands (1941), Allard (1949), Roemer (1942) and Anon. (1935).

In conclusion emphasis must be given to the importance of regular surveys made in conjunction with any breeding programme in order to detect new races as soon as they occur. Even if little is known regarding their origin, disease races may readily be introduced from other countries. It has been shown that Races 3 and 4 are typical of New Zealand, a country which imports wheat for blending either from Canada which has Races 1, 2, 3, 4 and 5, or from Australia where Phipps, Hockley & Pugsley (1943) have described Race 1. Such introductions are by no means unlikely, in view of Cherewick's (1944) evidence that perithecia may remain viable for 2 years.

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(*Received* 24 *February* 1950)

STUDIES OF THE CLOVE TREE

III. THE EFFECT OF THE SUDDEN-DEATH DISEASE ON WATER RELATIONS

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(With 1 Text-figure)

Extensive root disorganization is associated with all recognizable stages of the sudden-death disease of cloves, the final symptoms being those of a rapid wilt. In the early stages of the disease, however, the water status of an affected plant is more favourable than that of a healthy one, in spite of the root disorganization. In the diseased plant, the transpiring power and assimilation rate of the leaves are greatly lowered, probably because of partial closing of the stomata. The consequent reduction in transpiration appears to account for the smaller water deficit found in the earlier stages of the disease.

These results are considered to furnish additional evidence in favour of the hypothesis that the disease is caused by a pathogen.

Phenomena associated with the sudden-death disease, reported in the first paper of this series by Nutman & Sheffield (1949), point to a disturbance in the water relations of the tree as the immediate cause of death. From the available evidence it seemed likely that the root disorganization, which is present from a very early stage, restricts absorption, resulting in a gradually increasing water strain and leading to degeneration of the leaves, wilt and death. This possibility has now been investigated from a physiological point of view and the results here presented show this sequence to be most unlikely.

Studies of water deficit in relation to disease

An approximation to the water deficit of the leaves was obtained as follows. Leaves were picked from healthy and from suspect* trees. These were selected in pairs, one from each type of tree, and every endeavour was made to see that the members of each pair of leaves were of similar age and similarly exposed to incident radiation. Each leaf was at once punched with an identification mark, weighed on a torsion balance to the nearest milligram, and placed with its petiole dipping into water in a tin. This could be done in 15–20 sec., and weight changes over this period were imperceptible.

* As explained in a previous paper (Nutman & Sheffield, 1949), there are no symptoms known to be specific to the disease. The use of the word 'suspect' therefore needs definition. It refers to a stage considerably previous to the final wilt, but means that an experienced observer is reasonably certain that the tree is affected. 'Early suspect' implies, not only an earlier stage, but also some doubt of diagnosis.

Twelve leaves from each tree comprised the usual sample. The samples were brought to the laboratory and reweighed the next day, after they had had the opportunity of becoming fully imbibed in the dark and in a saturated atmosphere. Results are given as the change in weight expressed as a percentage of the fully imbibed weight. Table 1 illustrates a typical set of records.

TABLE 1. *Water deficit in clove leaves, as percentage difference between leaves when picked and when fully imbibed. Chunguziko, Pemba*

Leaf number	Tree I, healthy	Tree II, suspect	Tree III, healthy	Tree IV, suspect	Tree V, healthy	Tree VI, suspect
1	2·04	0·44	1·35	0·75	2·94	1·46
2	1·91	0·00	2·50	0·23	2·33	1·85
3	1·60	0·73	6·09	0·38	1·52	2·54
4	2·65	−0·26	1·96	0·42	2·84	2·34
5	2·74	0·73	3·66	0·61	3·67	2·25
6	4·12	0·32	3·91	0·85	3·37	3·07
7	2·54	0·00	5·48	0·34	2·91	1·84
8	4·91	0·32	3·10	1·69	2·13	2·46
9	2·30	0·92	3·92	1·11	3·86	3·32
10	1·77	1·06	3·84	0·76	4·99	3·48
11	2·94	1·43	5·61	1·75	5·18	2·90
12	5·17	0·44	2·96	3·72	4·20	1·86
Mean	2·89	0·51	3·70	1·05	3·33	2·45

Note. Trees V and VI were investigated during a period when radiation was considerably higher than when trees I–IV were being studied.

Statistical analysis shows that:

(*a*) The healthy trees do not differ significantly from each other.

(*b*) The two suspect trees under moderate light do not differ from each other but show a significantly *lower* water deficit than the healthy trees.

(*c*) The healthy tree under high radiation differs from the suspect tree under high radiation.

Summarized data from other areas are given in Table 2. They include trees in all stages from early suspect to final wilt.

Further evidence that, in the early stages of the disease, sudden-death does not increase the water strain is afforded by studies of the effect of the disease on the water content of leaves and branches. Diekmahns (1948) sampled leaves from healthy and diseased trees, each sample comprising one disk 1 cm. in diameter from each of ten leaves selected at random. Sixty-eight samples were taken, but no significant differences in water content between healthy and diseased leaves, whether measured at 10 a.m. or 2 p.m., were found.

Robb (1948) sampled the wood of nine trees, four healthy, three suspect and two in the early wilt stage, to a total of sixty sub-samples. Analysis showed there to be no significant difference between the water content of the wood of healthy and of suspect trees. The early wilt trees had, however, a significantly lower content.

The evidence is therefore clear that there is no reduction in the water content of leaves or branches, and no increase in the water deficit of the leaves, in the early stages of the disease. These results show that, in direct contradiction to previous views (Campbell, 1940), the water balance is more favourable in a suspect than in a healthy tree.

TABLE 2. *Water deficit, expressed as percentage difference between leaves when picked and when fully imbibed, in relation to disease*

District	Stage of disease	Average deficit
Mkaji	Healthy	4·31
	Early suspect	2·59
Mbuzini	Healthy	3·10
	Early suspect	0·96
Selem	Healthy	2·97
	Early suspect	2·60
Salaya	Healthy	3·41
	Suspect	2·21
Walezo	Healthy	3·73
	Suspect	0·17
Kazole	Healthy	1·93
	Suspect	0·89
Selem	Healthy	2·71
	Early wilt	3·80
Selem East	Healthy	4·61
	Early wilt	5·14
Selem	Healthy	2·52
	Early wilt	7·86

	Healthy	Suspect	Wilt
Mean	3·26	1·57	5·60

STUDIES OF TRANSPIRATION IN RELATION TO DISEASE

The somewhat unexpected nature of the results, so far described, might be explained, were the transpiration rates of healthy and diseased trees widely different. Studies of transpiration rates were therefore undertaken.

The only practicable method of comparing transpiration rates of diseased with healthy clove leaves under field conditions is by the use of the cobalt chloride technique. A slip of dried filter-paper, previously impregnated with cobalt chloride, is placed, under glass, against the transpiring surface of the leaf. The time necessary for the paper to change colour between two arbitrary standards is measured, and compared with the time necessary for a similar colour change when the paper is exposed at a standard distance from a free water surface. The ratio is the foliar transpiring power. This does not, of course, represent transpiration, but it is a measure of the potential transpiration rate. It is legitimate to assume that two

leaves, showing different transpiring powers by this method, would at a given time transpire at rates proportional to these transpiring powers.

The method can, of course, be applied to individual leaves only. Two trees can be compared by testing leaves alternately from each tree, care being taken to select similar leaves, hanging at the same angle to the incident light. Pairs of trees, one healthy and one suspect, were studied. Table 3 presents typical results, each entry representing the average of at least six pairs of determinations.

TABLE 3. *Comparison of foliar transpiring power of leaves from healthy and from diseased cloves*

District	Condition of tree	Test time (sec.)	Standard (sec.)	Transpiring power
Kazole	Healthy	25·8	20	0·77
	Suspect	74·5	20	0·28
Mkaji	Healthy	26·0	20	0·77
	Suspect	161·6	20	0·12
Selem	Healthy	73·0	34	0·47
	Suspect	119·1	34	0·29
Selem East	Healthy	66·4	34	0·51
	Suspect	160·9	34	0·12
Salaya	Healthy	22·2	20	0·90
	Suspect	62·6	20	0·32
Chunguziko	Healthy	66·8	34	0·51
	Suspect	119·3	34	0·21

Mean healthy, 0·66
Mean suspect, 0·24

This reduction in the transpiring power of the suspect tree is obviously of considerable significance. Since the records summarized above show the difference to be independent of external conditions, it seems very probable that there is some intrinsic difference between healthy and suspect trees. The series of records shown in Fig. 1 support this. They extend from morning until mid-afternoon, save for a rainy period at midday. Measurements were made on leaves from four trees, two healthy and two suspect. The trees were visited in rotation, one leaf from each being measured to a total of sixteen (four from each tree). This was repeated throughout the day. Statistical analysis of the detailed records shows that neither the two healthy trees nor the two suspects differ significantly from each other in transpiring power, but the healthy trees differ from the suspects. These results are in agreement with those of Glover (1939), who measured the transpiration rates of cut shoots from healthy and diseased trees. He found that of the diseased to be much lower, although, under the conditions of the experiment, both had equal opportunities to absorb water.

It is thus evident that the rate of water loss from a diseased leaf is appreciably lower than from a healthy one. This is probably true under all conditions, and may

well explain the improved water balance of the suspect tree, the reduction in transpiration rate more than compensating for the probable reduction in the absorbing power of the roots.

There are several possible causes for reduced rate of water loss, such as water tension in the plant (Haines, 1936), changes in protoplasmic permeability (Henderson, 1926) or changes in stomatal aperture. It cannot, in fact, be due to water tension, since increased water strain is demonstrably absent. Glover's results might be interpreted as being the result of increased resistance to water flow in the xylem, had not Diekmahns (1948) shown that there is no difference in this respect between the wood of healthy and of suspect trees.

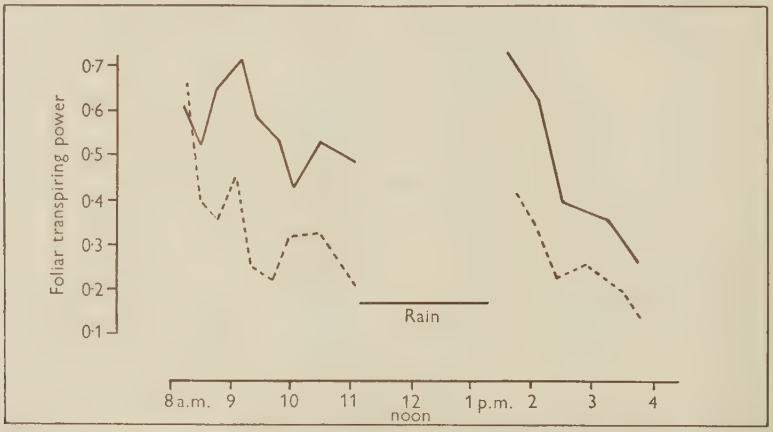

Fig. 1. The diurnal march of foliar transpiring power of healthy clove trees (entire line) and of suspect trees (dotted line). Chunguziko, Pemba.

Stomatal behaviour in relation to disease

The structure of the clove leaf makes investigation of stomatal movements and apertures very difficult, for it has a thick cuticle, few and small intercellular spaces and numerous but deeply sunken stomata (Sheffield, 1950). The only technique applicable is the infiltration method. Although this is admittedly crude, it can give roughly comparable results and can be used where the changes or differences under investigation are very large.

Using this method, the relative stomatal apertures of healthy and suspect trees were compared. Trees were selected in pairs as before and a circuit was made of each, ten leaves being tested from each tree. A drop of either alcohol or xylol was applied to the underside of each leaf, and the results recorded as the number of leaves showing evidence of infiltration. Table 4 presents results from eleven districts covering a wide area in Zanzibar Island.

TABLE 4. *Relative stomatal apertures of healthy and diseased clove trees, using the infiltration method, ten leaves being selected from each tree*

District	Total no. of trees used	Percentage of leaves injected	
		Healthy	Suspect
Walezo	10	78	20
Kianga	6	93	12
Kizimbani	6	70	7
Mdo	2	70	70
Mkanyageni	8	68	18
Kitundu	6	90	37
Salaya	10	96	14
Mkaji	8	75	25
Kazole	8	85	13
Kitope	4	95	5
Mbaleni	4	65	10
	Mean	81	21

It is clear that the stomatal aperture is greatly reduced in a diseased tree and this, in a very heavily cutinized leaf such as the clove, where most of the gaseous exchange must take place through the stomata, would seem an adequate explanation of reduced transpiration rate.

Additional confirmation can be obtained from measurements of photosynthesis in healthy and suspect leaves. Assimilation in tropical evergreens is often profoundly affected by stomatal movement, and Griffith (1946) has shown the rates of growth of a number of tropical timber trees to be correlated with an index derived from stomatal frequency and average stomatal aperture, while Nutman (1937) has presented evidence in favour of the view that stomatal control of assimilation is effective in *Coffea arabica*. Thus, reduced assimilation rates in suspect leaves would be expected, were stomatal control effective.

Diekmahns (1948), using the half-leaf method, studied the assimilation rates of samples of leaves from nineteen healthy and eighteen suspect trees over the period 10 a.m. to 2 p.m. The calculated rates for apparent assimilation, based on weight changes in disks 1 cm. in diameter, were: healthy leaves, 9·25 mg. dry matter/dm.^{2}/hr.; suspect leaves, 0·83 mg. dry matter/dm.^{2} hr. The difference is large and highly significant. It cannot be accounted for by loss of chlorophyll associated with chlorosis, since at this stage of the disease, chlorosis is not marked and measurements of chlorophyll content show only a slight decrease. Since chlorophyll is normally in excess, it seems highly probable that the changed assimilation rate associated with disease is due to stomatal changes.

DISCUSSION

The early effects of the sudden-death disease can now be defined more accurately than has hitherto been possible. By the time that the tree can be classed as a suspect, root disorganization is well advanced and absorption of water must be considerably

restricted. At this stage, changes in the leaves can be demonstrated, transpiration and assimilation both being greatly reduced. These changes in the leaves cannot be the result of decreased absorption, since the water deficit does not rise and they are almost certainly the result of stomatal closure. It now seems evident that the changes in the leaf are an immediate result of the disease.

It is even possible that the disorganization of the root system may be the direct consequence of the leaf changes. The clove is notably deficient in reserve carbohydrates (Campbell, 1940), and any appreciable reduction in carbon assimilation might well result in the loss of absorbing roots through starvation, for Storey (1939) has shown that starvation of the roots by ring-barking results in the death of the tree in a manner similar to sudden-death. On the other hand, the effects of the disease on root and shoot may be independent.

The evidence here presented has also some bearing on the cause of the disease, which has already been shown to be almost certainly either a virus or a fungus (Nutman & Sheffield, 1949), and enables us to restrict the possible causes still further. If root disorganization were due to the direct attack of a pathogen, the sequence leading to the death of the tree would necessarily involve increasing water strain from a comparatively early stage, and under no circumstances could a more favourable water balance result, whereas the increased water strain in the sudden-death disease is not evident until just before the final wilt. The results could, however, be explained, were the causal agent either a toxin-producing fungus or a virus.

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(*Received* 21 *February* 1950)

OBSERVATIONS ON NARCISSUS LEAF SCORCH IN SOUTH-WEST ENGLAND

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Narcissus leaf scorch caused by *Stagonospora Curtisii* (Berk.) Sacc. is very common in south-west England, where it causes economic damage arising from flower spot and leaf decay. Information is given as to the varying susceptibility of many different varieties, a comparative measure of which is obtained by counting the primary infections.

No certain control measures can be recommended, but results of experiments on bulb disinfection and on spraying are given.

The leaf-scorch disease caused by the fungus *Stagonospora Curtisii* (Berk.) Sacc. has been briefly referred to in many articles on narcissus diseases, and fully described by Creager (1933) and Moore (1939). Many observations and experiments have been carried out on this disease in south-west England, since the disease was first recorded there in 1925, and it is the object of this paper to give a brief account of data obtained supplementing those already published.

Life history

Early stages

Leaf-scorch disease may appear when bulbs are cleaned and planted in an isolated field, which has never grown narcissi before. This suggests that the fungus must be present either inside the bulb or on its surface. I have never seen pycnidia or mycelium on or between the scales in the dry bulb, but when bulbs are kept very moist the typical mycelium of the fungus may occasionally grow out from the browned tissues of the neck.

The fungus has been isolated from bulbs in a number of cases, generally from diseased tissues of the neck, but once from a rot of the outer scale. Considering the abundance of the disease, it is remarkable how rarely the fungus does appear in bulb isolations, for example only once did it appear in a very large number of isolations for Fusarium bulb rot.

When the shoot emerges from the bulb, infection by mycelium from the neck presumably takes place. The development of the tip lesions at this early stage is exceedingly slow. For example, a plot of Golden Spur in which the shoots emerged in January 1930 showed yellow blistered leaf tips on 13 March, which developed into typical tip scorch on 24 March. In general, from 3 to 5 weeks are required for the reddish yellow leaf tips to develop into typical leaf scorch. This slow develop-

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ment is probably related to the low temperatures ruling out of doors. In forcing boxes covered with straw typical primary leaf-scorch symptoms are normally visible as soon as the plants are put in the houses at the beginning of December.

Plants of the Polyanthus group normally emerge through the soil much earlier than the other groups, and the leaf-scorch disease develops on them earlier. For example, Double Roman has been seen with much leaf scorch in early November, and secondary infections may occur in a wet autumn resulting in severe leaf decay in January before flowering is complete. On the more commonly grown Soleil d'Or and Scilly White, leaf scorch is normally present in December and January. The almost equally susceptible Poetaz varieties, e.g. Medusa and High White, also often show severe tip scorch in December and January.

Pycnidia may be found on the scorched tips as early as January, but are not very common so early, except on Polyanthus varieties in Scilly and West Cornwall. Slugs commonly feed on *Stagonospora* lesions and the spores have been recovered from their faeces.

Flower spotting

As a general rule secondary infections do not begin until milder showery weather prevails in the early spring. In some seasons this may occur in March, but the great bulk of secondary infection occurs in April, at which time the fungus spreads on to the flowers as well as the leaves. The exact period in relation to the opening of the flower buds may make all the difference between a clean and an unmarketable flower crop. Two examples will make this clear. A crop of Sunrise in West Cornwall in 1941 was severely infected at the beginning of March, but the buds on this variety are normally carried up on long stalks. These buds were a couple of inches above the leaf tips by the time the lesions had begun to produce spores and the bulk of the flower crop was marketed with very little loss. Again in 1940, at Seale-Hayne College, Medusa and Ideal were grown side by side. There were many leaf lesions in the Medusa, but few on the Ideal. The tall stalks of the Medusa carried the flowers out of range of infection, but the short-stalked Ideal came into contact with the Medusa foliage and the flowers became badly infected.

Later varieties do not, as a rule, escape infection in this way, particularly Cheerfulness and most of the Poeticus group. In these the flower bud is often just below the leaf-tips at the period of maximum spore formation, resulting in severe losses from flower spot, unless the weather is dry. In dry weather the spores remain in the pycnidia, as rain is necessary for the dispersal of the pycnospores. It will thus be realized that the severity of an epidemic in any given season depends not only on the amount of wet weather, but also on its incidence in relation to the critical periods of flower development. A corollary of this is that any hope of controlling the disease by spraying will depend on correct timing of the spray application.

The economic damage from flower spotting is very considerable, and this is the phase of the disease that causes the greatest direct loss to the flower grower. Not only are flowers unmarketable because of spotting of the perianth segments and

brown decay of the spathe and pedicel and lesions on the flower stalks, but the disease may develop on the cut flowers in transit to market. These spots arise from the spores present on the flower surface, at the time of packing. Losses from flower spot are also caused by the fungi, *Botrytis narcissicola* and *B. polyblastis*, and it is often not possible to identify them with certainty without making isolations. Stagonospore flower spot is probably not so extensive as the *Botrytis* flower spots, but certainly causes local severe losses in many seasons.

Leaf decay

Later in the season plants bearing a number of leaf lesions, particularly those suffering from much secondary infection, become subject to premature leaf decay. This is not as extensive or as severe as that produced by narcissus fire or narcissus white mould, but local epidemics often develop, particularly in the more susceptible varieties. Gregory (1940) showed that white mould epidemics may result in losses in the weight of bulbs lifted after 3 years of as much as 44% and a corresponding reduction in the flower crop in each succeeding year. There are no similar data for the leaf-scorch disease, but it is not likely that the losses are so great.

Leaf decay occurs earliest on the Polyanthus varieties. It has been observed in Double Roman in January and is common in Scilly White and Soleil d'Or from March onwards, but on most varieties there is very little leaf decay until May.

Premature leaf decay is greatest in wet, badly drained soil and in beds which are 3 years old or more. It is very rarely seen in first-year beds. For example, out of sixty-seven bulbs of Seagull planted in 1935, only five plants were noted affected with leaf scorch in 1936, and there was no leaf decay. In 1937, every plant had from one to three leaves affected in February and by 23 April there was extensive leaf decay. In most seasons such a severe infection is not observed till the third year. This increase in leaf disease is not solely due to the increase in the number of leaves, as it may occur in varieties such as Soleil d'Or, in which it frequently happens that there is a decrease in the number of leaves. The following table gives one example of observations made at Newton Abbot, Devon:

Soleil d'Or planted 1941 (*front row, 52 rounds*)

	1942	1943
Percentage of plants infected	40	80
Percentage of leaves infected	7	30
Mean number of leaves per plant	8·0	6·5

Even when the leaves are completely decayed the flower stalks as a rule stand up green and are very often able to ripen any seed pods they may carry, but in one such case observed in early June in King Alfred, lesions due to the *Stagonospora* fungus just under the seed pod caused the pod to wither.

Susceptibility of varieties

The Polyanthus group is the most susceptible, but most of the varieties in the Leedsii, Poeticus and Poetaz groups are very susceptible. The Trumpets and Incom-

parabilis are much less susceptible, and economic damage is generally insignificant. Barrii varieties and Jonquil hybrids occupy an intermediate position.

The following lists name the more susceptible varieties, arranged according to the classification recently adopted by the Royal Horticultural Society:

Very susceptible	Moderately susceptible
—	**Division 1 (Trumpets)**
	Golden Spur
	Division 2 (Large-cupped narcissi)
Macebearer	Bonaparte
Lucifer	Croesus
Bernardino	Czarina
—	Kingdom
—	Lord Kitchener
—	Market Gem
—	Phyllida
—	White Nile
—	White Queen
	Division 3 (Small-cupped narcissi)
Evangeline	Albatross
Katherine Spurrell	Bath's Flame
Queen of the North	Brilliancy
St Olaf	Fair Maiden
White City	Seagull
White Lady	Sunrise
	Division 4 (Doubles)
Cheerfulness	Inglescombe
Double Roman	Mary Copeland
	Division 7 (Jonquilla hybrids)
—	Buttercup
—	Lanarth
	Division 8 (Tazetta)
	A. Polyanthus group
Soleil d'Or	Compressa
Grand Primo	—
Scilly White	—
	B. Poetaz group
Medusa	—
Glorious	—
Early Perfection	—
Orange Cup	—
High White	—
Ideal	—
St Agnes	—
Scarlet Gem	—
Sycamore	—
	Division 9 (Poeticus)
Cassandra	Dactyl
Dulcimer	Sarchedon
Homer	—
Horace	—
Juliet	—

Division 10 (Species)	
Poeticus var. *ornatus*	*Telamonius plenus*
var. *ornatus maximus*	*Jonquilla*
var. *recurvus*	*Odorus*
var. *flore pleno*	—
Biflorus	—
Tazetta var. *papyraceus*	—

The disease has been found on wild plants of the common Lent lily, *Narcissus pseudonarcissus*, and the fungus isolated from the affected leaves.

The only other host plant on which the disease has been found in the south-west is the Belladonna lily (*Amaryllis Belladonna*). This is exceedingly susceptible, and every stock examined in the south-west is affected.

Measurement of susceptibility

The concept of susceptibility is a complex one and really covers two types: (*a*) individual susceptibility, (*b*) epidemic or field susceptibility. The former is the reaction of an individual plant to inoculation by the fungus, and might be measured by the percentage of infection resulting from inoculations carried out under optimum conditions. The second is the more practically useful conception of the amount of disease that develops in different varieties when subject to similar conditions, and takes into account the varying rate of spread within a stock of plants as well as the individual susceptibility of each plant. In practice, narcissus varieties differ little in individual susceptibility, all readily taking the disease when inoculated. But the growth and spore production on the plant vary greatly and therefore also the rate of spread and consequently the field susceptibility.

An attempt has been made to get a measure of varietal field susceptibility by counting the number of primary infections. A large stock of Golden Spur available in 1934 gave the opportunity of testing the uniformity of the results (Table 1). All the bulbs in lots 1–4 were planted in forcing boxes on 5 September 1934, and those in lots 5 and 6 were planted a month later. All the bulbs were given 1 hr. hot-water treatment on 8 August, except lot 1.

Table 1

Lot	Number of plants	Percentage of plants showing *Stagonospora* infection
1	466	8
2	340	4
3	317	9
4	317	6
5	197	20
6	399	15

It may be concluded that when the growth conditions are uniform the measurements are comparable, but as would be expected, differing growth conditions such as date of planting, soil moisture, etc., will bring out differences in the results. In this experiment the later planting more than doubled the amount of leaf-tip infection.

In the following experiment, bulbs grown at the same place were planted in forcing boxes in October 1941, and counts were made in the glasshouse in March 1942. The percentages of primary infections were as follows:

Polyanthus group	%	Other groups	%
Grand Monarque	6	Cicely	5
Compressa	0	Princess Victoria	0
Poeticus group		Hospodar	0
Horace	8	Helios	0
Homer	12	Minister Talma	0
Poetarum	18	M. J. Berkeley	0
Epic	15		

These results confirm general field observations, and in particular bring out the resistant quality of Compressa which is unusual for its group.

CONTROL

Theoretically the following measures might give some control of the leaf-scorch disease:

(1) Bulb disinfection to check primary infection of the shoot.
(2) Leaf-tip cutting to remove the primary infections.
(3) Spraying to prevent secondary infections.
(4) Cutting off foliage to prevent reinfection of the bulb.

Practical experiences in the south-west have given reliable results only with the second method, though sometimes spraying has contributed a measure of control.

Spraying with Bordeaux mixture, as recommended by Gregory (1940), is carried out by a number of growers in Devon and Cornwall, and good results have been observed in the reduction of *Stagonospora* leaf decay. For example, at Bere Ferrers in 1940 one bed of Cheerfulness which had been sprayed stood out quite green in marked contrast to the other beds left unsprayed.

Creager (1933), Haasis (1934) and McWhorter & Weiss (1932) claim a reduction in the amount of primary infection by immersion of the bulbs in formalin or in mercuric chloride immediately before planting. My experiments with Soleil d'Or and Macebearer with $\frac{1}{4}\%$ formalin and two proprietary organic mercury disinfectants have given negative results, as did also experiments on cutting off Macebearer foliage early in June.

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(*Received* 18 *March* 1950)

THE USE OF PROBITS IN COMBINING PERCENTAGE KILLS

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(With 1 Text-figure)

The problem is discussed of how to combine percentage kills obtained in an experiment repeated under different sets of conditions. Treatment effects and their interaction with replicates are defined in terms of probits, and, assuming the absence of such interaction, a method is evolved of finding maximum likelihood estimates of the mean treatment differences. An example is used to illustrate details of the method. The merits of the logit and angular transformations applied to the same type of data are also discussed.

The problem

In the case of a replicated experiment in which the responses are measured in terms of a normally distributed variate, the information from the separate replicates can be pooled by a simple additive process. The resulting weighted or unweighted totals (or means) are normally distributed with an estimable variance, and the analysis of variance technique provides valid tests of significance on the average treatment responses.

The corresponding problem when the responses are measured in terms of the proportion of individuals reacting to the treatment, such as percentage kill in toxicity tests, is somewhat more complicated. If it is assumed that each replicate of the test was carried out under identical conditions, the numbers killed are clearly additive. Such an assumption, however, is seldom justified. The mean percentage kill over all treatments may differ considerably from one occasion to the next, and any attempt to combine the information in a single contingency table by straightforward addition of the numbers killed will both invalidate χ^2 tests on the resulting totals and render interpretation wellnigh impossible. Thus, some other method must be sought which will meet both these difficulties. That is, we wish to discover some sort of 'average' treatment effect to which a significance test can be applied, while the corresponding 'average' kills should represent, if possible, the kills expected under some intermediate set of conditions.

Interaction

If the relative treatment effects (not yet defined) are markedly different in the different replicates we may rightly query the advisability of combining the information, but, if these differences are assumed negligible, mean responses may be estimated for the purpose of comparing treatments.

The first step is to define interaction of treatment differences with replicates. Bartlett's (1935) definition of interaction in a $2 \times 2 \times n$ contingency table gives as the condition for absence of interaction

$$\frac{p_1 q_2}{q_1 p_2} = \text{constant}$$

for all treatments, p being the proportion responding to the treatment, $q = 1 - p$, and suffixes referring to replicates.

Equally, we may introduce the concept of parallel probit lines, giving the condition

$$Y_1 - Y_2 = \text{constant},$$

where Y_1 and Y_2 are the probits corresponding to p_1 and p_2. This is the convention which we shall adopt for the present. It is a condition that may be expected to hold over a wide range of experimental results, and one that leads to a satisfactory method of analysis. This and Bartlett's definition are synonymous only when p_1 and p_2 are both around 50%. However, if in making a rough test for absence of interaction Bartlett's condition is used for convenience, no very large errors will be committed provided p_1, q_1, p_2, q_2 all lie between about 10 and 90%.

Having thus defined interaction, treatment differences can now be expressed as the distances between the true probit lines joining probits of like treatments. In other words, if two tests are made with each of two treatments A and B, and there is no interaction, then the relative treatment effect is measured by either

$$Y_{1(A)} - Y_{1(B)} \quad \text{or} \quad Y_{2(A)} - Y_{2(B)}.$$

The maximum likelihood equations

The problem is now reduced to one of fitting a system of parallel probit lines to the general case of t treatments with k replicates. It will be convenient to use biological terminology throughout.

Let us assume the existence of some parameter β, constant for any one replicate (or occasion), and associated with the variation in the average mortality between occasions. Further, since the scale of measurement of β is purely arbitrary we may choose this scale such that for any given treatment Y and β are linearly related, and $dY/d\beta = 1$. Thus, on the assumption of no interaction, the expected probit Y is given by

$$Y = \alpha + \beta,$$

where α is constant for a given treatment, differences between the α's representing differences in treatment effects. Both the α's and the value of β for each occasion will require to be estimated from the data, and for this the maximum likelihood method (Kendall, 1945) may be used.

If n is the number of insects in a batch, P the expected proportion killed and $Q = 1 - P$, the probability of getting r deaths is

$$\binom{n}{r} P^r Q^{n-r}.$$

The likelihood function L is therefore given (apart from constants) by

$$\log L = Sr \log P + S(n-r) \log Q,$$

where S denotes summation over all batches, and if θ is any parameter to be estimated from the data, the maximum likelihood estimate of θ is derived from the equation

$$\frac{\partial \log L}{\partial \theta} = 0.$$

Now

$$\frac{\partial \log L}{\partial \theta} = S \frac{n(p-P)}{PQ} \frac{\partial P}{\partial \theta},$$

where $p = r/n$. Hence, if α_i refers to the ith treatment, β_j to the jth occasion, and $S_{i.}$, $S_{.j}$ and $S_{..}$ represent summations over β for a given treatment, over treatments for a given occasion and over all the observations respectively, the α's and β's are estimated from the set of simultaneous equations:

$$S_{i.} \frac{n(p-P)}{PQ} \frac{\partial P}{\partial \alpha_i} = 0 \quad (i = 1, 2, \ldots, t),$$

$$S_{.j} \frac{n(p-P)}{PQ} \frac{\partial P}{\partial \beta_j} = 0 \quad (j = 1, 2, \ldots, k).$$

If Z be the ordinate of the normal distribution corresponding to Y, then $dP/dY = Z$, which, since $Y = \alpha + \beta$, gives the relations

$$\frac{\partial P}{\partial \alpha} = \frac{\partial P}{\partial \beta} = Z,$$

P, α and β having the values corresponding to the set of observations in question. Further, we may, following the methods of probit analysis (Bliss, 1935; Finney, 1947*b*), define the working probit, y, by

$$y = Y + \frac{p-P}{Z},$$

and the weighting coefficient, w, by

$$w = \frac{Z^2}{PQ}.$$

Using these relations, the maximum likelihood equations reduce to

$$S_{i.} nw(y - Y) = 0 \quad (i = 1, 2, \ldots, t),$$

$$S_{.j} nw(y - Y) = 0 \quad (j = 1, 2, \ldots, k),$$

and on substituting $Y_{ij} = \alpha_i + \beta_j$ and denoting by $\bar{y}_{i.}$, $\bar{\beta}_{i.}$, etc., the weighted means $\frac{S_{i.} nwy}{S_{i.} nw}$, $\frac{S_{i.} nw\beta}{S_{i.} nw}$, etc., we obtain

$$\left.\begin{aligned} \alpha_i &= \bar{y}_{i.} - \bar{\beta}_{i.} \quad (i = 1, 2, \ldots, t), \\ \beta_j &= \bar{y}_{.j} - \bar{\alpha}_{.j} \quad (j = 1, 2, \ldots, k). \end{aligned}\right\} \tag{1}$$

Of these $t + k$ equations $t + k - 1$ are independent, so that by imposing one further restriction—which amounts to choosing an origin—unique solutions for the parameters α and β can be found.

In practice, solutions are most easily obtained by successive approximation. First, suppose that the true values of Y are replaced by first approximations and the corresponding working probits and weighting coefficients found from the tables of Fisher & Yates (1948). Since differentiating y with respect to Y gives

$$\frac{dy}{dY}=(Y-5)\frac{p-P}{Z},$$

which will be small due to the factor $(p-P)$, these working probits will be good approximations to the true working probits which would be obtained if the true values of Y were known. If first approximations of the β's are also available, values for the α's can then be found by substitution in the first group of equations (1). Substitution in the second group of equations (1) then provides better estimates of the β's, which in turn can be used to give improved estimates of the α's. One cycle will generally give the required degree of accuracy in the α's provided that the first approximations are reasonably good.

These initial approximations may be derived in any convenient manner. The simplest method is to replace the weighted means in equations (1) by unweighted means, using the observed probits in place of working probits. Denoting unweighted means by double bars and choosing the origin such that $\bar{\bar{\beta}}_{i.}=\bar{\bar{\beta}}_{..}=0$, this gives the following first estimates for the β's and the Y's:

$$\beta_j=\bar{y}_{.j}-\bar{\bar{\alpha}}=\bar{\bar{y}}_{.j}-\bar{\bar{y}}_{..},$$
$$Y_{.j}=\alpha_i+\beta_j=\bar{\bar{y}}_{i.}+\bar{\bar{y}}_{.j}-\bar{\bar{y}}_{..}.$$

It is useful to remember that if an observed probit is reasonably close to its expectation, then $p-P$ is small and the observed will be practically equal to the working probit. If used with discretion this relationship will frequently enable working probits to be written down without referring to tables.

Tests of significance

If a number of mutually independent parameters, θ_1, θ_2, etc., are estimated by the maximum likelihood method, the variances and covariances of the estimates are given by the matrix:

$$\begin{pmatrix} -\frac{\partial^2 \log L}{\partial\theta_1^2}, & -\frac{\partial^2 \log L}{\partial\theta_1\partial\theta_2}, & \cdots \\ -\frac{\partial^2 \log L}{\partial\theta_1\partial\theta_2}, & -\frac{\partial^2 \log L}{\partial\theta_2^2}, & \cdots \\ \vdots & \vdots & \end{pmatrix}^{-1}.$$

In the present case any one of the parameters is dependent on the remaining $t+k-1$, and therefore one of these must first be eliminated. Clearly, since the choice of origin was arbitrary, we may in order to estimate the variances appropriate to treatment differences equate one of the β's to zero. Thus putting $\beta_k=0$ we obtain the above matrix with the row and column corresponding to β_k missing. On evaluating

the separate elements and inverting, it is found that the variances and covariances corresponding to the α's are given by the symmetric matrix

$$V = \begin{pmatrix} S_{1.} - \left\{\frac{(11)^2}{S_{.1}} + \frac{(12)^2}{S_{.2}} + \ldots + \frac{(1\,k-1)}{S_{.k-1}}\right\}, & -\left\{\frac{(11)(21)}{S_{.1}} + \frac{(12)(22)}{S_{.2}} + \frac{(1\,k-1)(2\,k-1)}{S_{.k-1}}\right\}, & \ldots \\ -\left\{\frac{(11)(21)}{S_{.1}} + \frac{(12)(22)}{S_{.2}} + \ldots + \frac{(1\,k-1)(2\,k-1)}{S_{.k-1}}\right\}, & S_{2.} - \left\{\frac{(21)^2}{S_{.1}} + \frac{(22)^2}{S_{.2}} + \ldots + \frac{(2\,k-1)^2}{S_{.k-1}}\right\}, & \ldots \end{pmatrix}^{-1}, \text{ etc.}$$

where $(ij) \equiv (nw)_{ij}$, i.e. the value of nw corresponding to the ith treatment on the jth occasion and $S_{i.} \equiv S_i . nw$, etc. If the elements of the inverted matrix are v_{rs} then the variances of the treatment differences are given by

$$V(\alpha_r - \alpha_s) = v_{rr} - 2v_{rs} + v_{ss},$$

and the appropriate significance tests are carried out using tables of the normal distribution (Fisher & Yates, 1948).

Inversion of the matrix, however, will in general be a troublesome affair, and the following approximate formula will give estimates of sufficient accuracy for most purposes:

$$\mathrm{V}(\alpha_r - \alpha_s) = \frac{1}{S_{r.}} + \frac{1}{S_{s.}} + \frac{1}{t}\left\{\frac{1}{S_{r.}} + \frac{1}{S_{s.}} - \frac{2\Sigma_j \surd[(nw)_{rj}(nw)_{sj}]}{S_{r.}\,S_{s.}}\right\}.$$

The part within the bracket will be small relative to the first two terms, and if, for each value of j, $(nw)_{rj} \doteq (nw)_{sj}$, this correction term may be neglected giving

$$V(\alpha_r - \alpha_s) \doteq \frac{1}{S_{r.}} + \frac{1}{S_{s.}}.$$

Since the correction term is essentially positive the last-mentioned approximation will tend to underestimate the true variance.

Heterogeneity

If the insects in a particular batch do not react independently of one another, or if interaction of treatments with replicates exists, the discrepancies between observed and expected probits will be unduly large. By comparing these discrepancies with those expected on the basis of purely random fluctuations in the behaviour of individuals, it is possible to test whether the above assumptions are, in fact, justified. Any such heterogeneity of departures from expectation will be indicated by a significantly high value of χ^2, where

$$\chi^2 = S_{..} nw(y - Y)^2$$
$$= S_{..} nw(y - \alpha - \beta)^2.$$

The appropriate number of degrees of freedom is $(t-1)(k-1)$.

EXAMPLE

In an experiment carried out at Rothamsted and designed to test the effectiveness of nicotine treatments on *Macrosiphum solanifolii* (*gei*) on strawberry an abstract of the results on three separate occasions gave the values of n and r shown in Table 1. The

TABLE 1

	17. xii. 45				30. i. 46			
Treatment	n	r	p (%)	Observed probit	n	r	p (%)	Observed probit
A	47	43	91	6·34	43	43	100	(6·72)
B	41	17	41	4·77	48	24	50	5·00
C	45	11	24	4·29	45	17	38	4·69
D	43	4	9	3·66	45	9	20	4·16
Mean probit (unweighted)				4·76				5·14
β'				−0·50				−0·12

	11. iii. 46				
Treatment	n	r	p (%)	Observed probit	Mean probit (unweighted)
A	45	45	100	(7·44)	6·83
B	45	35	78	5·77	5·18
C	46	33	72	5·58	4·85
D	43	16	37	4·67	4·16
Mean probit (unweighted)				5·87	5·26
β'				+0·61	

observed probits are found directly from the tables except in the two cases where $p = 100\,\%$. These must be treated for the moment as missing observations and their values estimated. Thus, neglecting treatment A, the means for the columns are respectively 4·24, 4·62 and 5·34. In the first column treatment A exceeds this mean by $6{\cdot}34 - 4{\cdot}24 = 2{\cdot}10$, which is the amount by which treatment A should exceed the corresponding means in the other columns. Hence the values 6·72 and 7·44. First approximations, β', of the β's are then evaluated as previously explained from the unweighted marginal means. Thus

$$\beta'_1 = 4{\cdot}76 - 5{\cdot}26 = -0{\cdot}50.$$

The first approximations, Y', to the expected probits are shown in Table 2, and are computed by adding in turn each β'_j to each of the unweighted means in the final column of Table 1. Thus

$$4{\cdot}68 = 5{\cdot}18 + (-0{\cdot}50).$$

Corresponding values of nw are also shown in Table 2.

Having thus obtained all the necessary first approximations the main part of the computations is carried out as illustrated in Table 3. The working probits forming the body of the table would in general be obtained by looking up probit tables under

the values of Y' in Table 2. In this example differences between observed and expected probits were considered small enough—except in the two instances involving 100% kills—to approximate the working to the observed probits. The largest error occasioned by this short-cut is 0·01.

TABLE 2

	17. xii. 45		30. i. 46		11. iii. 46		
Treatment	Y'	nw	Y'	nw	Y'	nw	$S_i.nw$
A	6·33	15·3	6·71	8·8	7·44	2·6	26·7
B	4·68	25·1	5·06	30·5	5·79	22·7	78·3
C	4·35	24·5	4·73	27·9	5·46	27·1	79·5
D	3·66	13·9	4·04	20·3	4·77	26·8	61·0
$S_{.j}nw$		78·8		87·5		79·2	245·5

TABLE 3. *Working probits*

Treatment	1st test	2nd test	3rd test	$\bar{y}_{i.}$	$\bar{\beta}'_{i.}$	$\bar{y}_{i.}-\bar{\beta}'_{i.}$ $=\alpha'_i$	$\bar{\beta}_{i.}$	$\bar{y}_{i.}-\bar{\beta}_{i.}$ $=\alpha_i$
A	6·34	7·18	7·80	6·76	−0·27	7·03	−0·28	7·04
B	4·77	5·00	5·77	5·15	−0·03	5·18	−0·03	5·18
C	4·29	4·69	5·58	4·87	+0·01	4·86	+0·01	4·86
D	3·66	4·16	4·67	4·27	+0·11	4·16	+0·11	4·16
$\bar{y}_{.j}$	4·73	4·93	5·40					
$\alpha'_{.j}$	5·26	5·03	4·79					
$\bar{y}_{.j}-\bar{\alpha}'_{.j}=\beta_j$	−0·53	−0·10	+0·61					

The next step is to compute the marginal means $\bar{y}_{i.}$ and $\bar{y}_{.j}$, using weights nw appropriate to each row or column. The column $\bar{\beta}'_{i.}$ is found from the weighted means of the β''s of Table 1, and the differences, $\bar{y}_{i.}-\bar{\beta}'_{i.}$, then give the next column α'_i, dashed letters throughout referring to first approximations. Again using weights appropriate to each column in turn, the weighted means $\bar{\alpha}'_{.j}$ of the α''s are written down under the marginal means $\bar{y}_{.j}$. The differences $\bar{y}_{.j}-\bar{\alpha}_{.j}$ then produce improved estimates of the β's, and on repeating the procedure by calculating the weighted means of these β's and subtracting as before from the $\bar{y}_{i.}$'s, we obtain improved estimates of the α's as shown in the final column of the table.

If more accurate estimates of the α's were required a further cycle of operations could be carried out on Table 3, but in the present case the resulting gain in accuracy is negligible. As a check on whether a further cycle is necessary, it should be sufficient to compute the next set of β's and verify that these have become moderately stable. It will seldom be worth while to use these estimates of α and β to obtain better approximations of the expected probits and hence of the nw's.

The fitted probit lines are shown in Fig. 1, observed probits being denoted by crosses. A mean value of the probit corresponding to the mean value of β, $\bar{\beta}_{..}$, can be calculated if desired for each treatment from the formula

$$\bar{Y}=\alpha_i+\bar{\beta}_{..},$$

$\bar{\beta}_{..}$ being equal to $-0{\cdot}02$. These means, together with the corresponding percentage kills $\bar{p}$, are shown in Table 4.

TABLE 4

Treatment	$\bar{Y}$	$\bar{p}$	$1/S_i.nw$
A	7·03	97·9	0·03745
B	5·17	56·8	0·01277
C	4·85	44·0	0·01258
D	4·15	19·8	0·01639

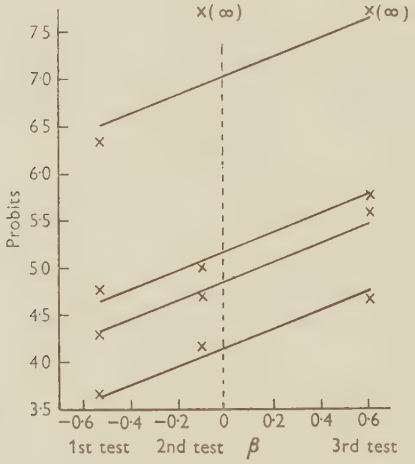

Fig. 1. Fitted probit lines for treatments *A*, *B*, *C*, *D* (Example).

As an example of a significance test let us consider the difference between, say, treatments *B* and *C*. We have from the variance formula

$$V(\alpha_B-\alpha_C)=0{\cdot}01277+0{\cdot}01258+\frac{1}{4}\left\{0{\cdot}01277+0{\cdot}01258-\frac{2\times 78{\cdot}77}{78{\cdot}3\times 79{\cdot}5}\right\}$$
$$=0{\cdot}02535+0{\cdot}00001$$
$$=0{\cdot}02536.$$

Therefore $\text{S.E.}(\alpha_B-\alpha_C)=0{\cdot}159.$

The correction term in this instance is negligibly small. The corresponding value of t is $\frac{5{\cdot}18-4{\cdot}86}{0{\cdot}159}=2{\cdot}01$, so that the difference is significant at the 5 % level, t being based on an infinite number of degrees of freedom.

The only satisfactory method of computing the heterogeneity χ^2 is to evaluate each $y_{ij}-\alpha_i-\beta_j$ and calculate the weighted sum of squares $S_{..}\,nw(y-\alpha-\beta)^2$. Any attempt to use an algebraically simpler formula results in considerable inaccuracy. This gives $\chi^2=2{\cdot}56$, based on 6 degrees of freedom, a value somewhat lower than we expect, showing that in this example the probit lines fit the data unusually well.

Other possible transformations

The choice of transformation depends entirely on the definition of interaction, and each transformation in turn implies a certain distribution of individual tolerances as measured on the transformed scale. If, for example, we adopt Bartlett's condition for absence of interaction, this leads immediately to the logit transformation, the parallel probit lines of the previous theory being replaced by parallel logit lines, where the logit, Y, is defined by

$$P = \frac{1}{1+e^{-2(Y-5)}},$$

or

$$Y = 5 + \tfrac{1}{2}\log\frac{P}{Q}.$$

The maximum likelihood solution is derived as before, and the same equations hold good, except that now

$$y = Y + \frac{p-P}{2PQ},$$

and

$$w = 4PQ.$$

Working logits and weighting coefficients have been tabulated as for probits by Finney (1947*a*). Alternatively, Fisher & Yates explain how their Table 7 (transformation of r to z) can be used for this purpose. The details of computation and evaluation of the heterogeneity χ^2 are exactly as in the probit case.

Another possibility is the angular transformation

$$P = \sin^2 Y.$$

Here the weighting coefficient is constant for all values of Y, being $\frac{1}{820{\cdot}7}$ if Y is measured in degrees or 4 if Y is in radians. In forming the weighted means, therefore, w can be omitted, and in fact need only be used in computing the variances and χ^2. The means have still to be weighted, of course, inasmuch as the number of individuals, n, will not in general be constant. Tables of the working angle

$$y = Y + \frac{p-P}{2\sqrt{(PQ)}}$$

are given in Fisher & Yates.

In general, there is little theoretical ground for preference as between probits and logits. The two concepts of interaction are not widely discrepant, and one may give as good a fit as the other. If tables of working logits are not available it will be quicker to use probit tables than to obtain logits from Fisher & Yates's $r-z$ table. Otherwise there is no difference in the amount of computation involved.

The disadvantage of the angular transformation is that it is likely to have a more approximate theoretical basis. Since to $P=0$ and $P=1$ correspond values of Y of 0 and 90° respectively the distribution of tolerances as measured on the Y scale is clearly a finite one. It is too much to expect that such a distribution is ever accurately

realized in nature, and we are therefore led to regard Y as an artificial symbol invented by the statistician for his own convenience rather than as the simple measurement of some quantitative character. This being so, it is unreasonable to believe that any simple law, such as that defined by absence of interaction, will be obeyed exactly. In the majority of cases the objection is probably not a serious one. Violent contradiction of the underlying hypotheses will as before be indicated by a large χ^2. There is a slight saving in labour by not having to look up weighting coefficients and a further saving if n is constant.

Table 5 shows the results of using logits and angles on the data of the example. Agreement of the mean percentages is very close in each case.

TABLE 5

	Logit			Angular		
Treatment	$\bar{Y}$	$\bar{p}$	$1/S_{i.}nw$	$\bar{Y}$	$\bar{p}$	$1/S_{i.}nw$
A	6·80	97·3	0·03846	83·1	98·5	6·08
B	5·16	58·0	0·00861	48·9	56·8	6·12
C	4·89	44·5	0·00855	41·9	44·6	6·03
D	4·29	19·5	0·01235	27·3	21·0	6·26
$\chi^2_{(6)}$		3·05			4·95	

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(*Received* 11 *January* 1950)

THE EFFECT OF VARIATIONS IN CALCIUM SUPPLY, pH VALUE AND NITROGEN CONTENT OF NUTRIENT SOLUTIONS ON THE RESPONSE OF LETTUCE AND RED CLOVER TO MOLYBDENUM

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(With Plates 16 and 17 and 2 Text-figures)

Lettuce and red clover were grown in nutrient solutions with varied calcium supply, pH value, and nitrogen content, and the response to molybdenum compared under each set of conditions.

The calcium requirement was greater in solutions at pH 4·4 than at 6·3, but the quantity of calcium supplied did not affect the response of the plant to molybdenum. Growth was best in the more acid of a range of solutions from pH 4·2 to 8·2 in spite of a rapid levelling up to a pH between 6 and 7, but with the possible exception of the solution at pH 8·3, the need for molybdenum was unaffected by the reaction of the medium. When the calcium supply and/or the initial pH value of the solution was varied, the effect of molybdenum was most pronounced in the largest plants.

When the nitrogen supply was deficient, lettuce showed a slower response to molybdenum than when it was plentiful. With both inoculated or uninoculated clover the reverse was true. This difference in behaviour is explained on the assumption that lettuce has a smaller requirement for molybdenum than clover.

In both lettuce and clover the percentage nitrate-nitrogen in the dry matter of the shoot was higher when molybdenum was not supplied, but the total nitrogen content was increased in the case of lettuce only. At any level of nitrogen supplied, 5 or 10 p.p.m. molybdenum was of no more benefit than 0·1 p.p.m. though the liability to damage from toxicity was possibly greater when nitrogen was plentiful.

Introduction

Molybdenum is now regarded as an essential element for the healthy growth of a number of crops, and the symptoms that appear when it is lacking are fairly well known.

Molybdenum deficiency has chiefly been reported for legumes on acid, iron-stone gravel soils in Australia and Tasmania (Anderson, 1942; Fricke, 1944). In some cases (Fricke, 1944), the addition of lime brought about an improvement in growth, a result attributed to an increase in the availability of molybdenum with the rise in pH. Lewis (1943) and Stephens & Oertel (1943) also found the element was more readily absorbed from alkaline soil. In other instances, no improvement in the crop resulted unless molybdenum itself were added. Benefit in either case usually appeared as a deepening in colour of the plant, and an increase in yield, but the two effects could occur independently (Fricke, 1944; Warington, 1945). The part played

by molybdenum in affecting this improvement in plant growth is not completely understood, but as far as leguminous plants are concerned, Jensen & Betty (1943) and Anderson & Thomas (1946) have shown that the element is needed for symbiotic nitrogen fixation by the nodule organism. A close association between molybdenum and nitrogen is also found in the lower plants, Bortels (1930) having shown it to be essential for nitrogen fixation by *Azotobacter*, and Steinberg (1936), that it is needed by *Aspergillus* for the reduction of nitrate. Mulder (1948) has largely confirmed Steinberg's results and shown that molybdenum is required for denitrification, as well as being necessary for nitrate reduction in higher plants of a non-leguminous type, such as tomato, oats and barley. That an association with nitrogen metabolism may not be the sole function of molybdenum is, however, suggested by other investigators. Working with flax in nutrient solutions, Millikan (1947) showed that addition of molybdenum could prevent the appearance of iron deficiency symptoms induced by excess manganese, zinc, copper, nickel or cobalt. From this he concluded that an essential function of the element is the regulation of the physiological availability of iron to the plant.

The response of the plant to molybdenum in both field and glasshouse experiments has proved to be variable. Seasonal conditions alone do not seem to account for this (Warington, 1945), though the demand of the plant probably differs with its stage and rate of growth. The temporary introduction of small quantities of the element from impurities or through its liberation by changes in soil conditions may be partly responsible for some of these inconsistencies, but the matter seemed to merit further study. Experiments were accordingly carried out in culture solutions during 1946–9, to see if alterations in the nutrient conditions would influence the response of the plant to molybdenum.

EXPERIMENTAL METHODS

Nutrient solutions and modifications

Two nutrient solutions, Rothamsted and Crone, were used, the chief difference between them lying in the form in which the phosphate and iron were supplied:

Rothamsted	(g./l.)	Crone	(g./l.)
KNO_3	1·0	KNO_3	1·0
$MgSO_4.7H_2O$	0·5	$MgSO_4.7H_2O$	0·5
KH_2PO_4	0·4	$CaSO_4.2H_2O$	0·5
K_2HPO_4	0·135	$Ca_3(PO_4)_2$	0·25
$CaSO_4.2H_2O$	0·5	$Fe_3(PO_4)_2$	0·25
$FeCl_3$ *or*	0·04	Both solutions H_3BO_3	
$FeC_6H_5O_7.3H_2O$	0·048	*and* $MnSO_4.4H_2O$	0·001

The nitrogen supply was identical. Small variations in the iron and calcium were sometimes made when neither was the factor under investigation. All salts, with the exception of the ferric chloride and citrate, were shown to be free of detectable amounts of molybdenum by spectrographic examination, and since plants supplied

with the samples used of both these iron salts had shown good symptoms of molybdenum deficiency, their purity was considered satisfactory. The pH value of the Rothamsted solution was approximately 5·4 or 5·7, according to whether ferric chloride or ferric citrate was used, while that of the Crone formula was usually about 6·4. Five replicates for each treatment were grown singly in glass vessels varying from 400 to 800 c.c. capacity and the solutions renewed fortnightly. Using these solutions as a basis, three factors, viz. calcium content, pH value and nitrogen supply, were varied, and the effect of a deficiency of molybdenum determined under each set of conditions.

Calcium content. Alterations in the calcium level were feasible in the Rothamsted solution only, since too many changes in other ions would have been involved with the Crone formula. Calcium chloride was substituted for calcium sulphate, for greater convenience and accuracy. Both salts proved equally satisfactory as a source of calcium.

pH *value.* Variations in pH value were made both with and without reductions in the calcium supply. Two methods were used, depending on the nature of the nutrient solution and the range of pH required. Provided a value of not much above 6·2 was wanted, the reaction of the Rothamsted solution (with ferric chloride) was most conveniently adjusted by altering the proportion of the two phosphates, KH_2PO_4 and K_2HPO_4, and this was the method employed when the calcium supply was also being varied. The formula, shown above (using ferric chloride), produces a solution of pH about 5·4; a combination of 0·45 g. KH_2PO_4 with 0·068 g. K_2HPO_4 one of pH about 4·4; while 0·3 g. KH_2PO_4 and 0·27 g. K_2HPO_4 are required for a solution of pH about 6·3. The P contents of these solutions was almost identical, but slight variation in the K supply was unavoidable. When extension into a more alkaline range was desired, ferric citrate was substituted for ferric chloride, and both in this case and in the Crone solution, pH adjustments were carried out by adding hydrochloric acid or sodium hydroxide. All pH determinations were made with a Hellige comparator and standard colour disks.

Nitrogen supply. Where alterations in nitrogen supply were made, potassium chloride was substituted for potassium nitrate, and the desired nitrogen added in the form of sodium nitrate.

Lettuce and red clover were selected as suitable crops, since both were known to require molybdenum, but their nitrogen nutrition, which was likely to be of special interest, differed.

Effect of alterations in the calcium

Supply and pH value in the presence and absence of molybdenum

In soil, the small quantities of molybdenum present are more available to the plant under alkaline than acid conditions. Some difference in uptake of molybdenum might therefore occur between plants grown in solutions of different pH value. The

appearance of molybdenum deficiency symptoms, on the other hand, should be unaffected by changes in the reaction of the culture solution, since here the medium is theoretically molybdenum-free.

(A) *Calcium supply and* pH *value both modified*

Lettuce

Three experiments were carried out in 1946, using Cheshunt Early Giant or Tom Thumb according to the season. The plants were grown in Rothamsted solutions containing 0·118, 0·030 or 0·015 g. Ca/l., each at a pH of approximately 4·4, 5·4 or 6·3. For comparison, similar plants were set up in Crone solution (pH 6·4), which provided nearly twice as much calcium as the normal Rothamsted formula, but in a less soluble form. All treatments were carried out with and without 0·1 p.p.m. molybdenum.

Results of treatment in the presence of molybdenum. As is commonly found, the pH value of the nutrient medium did not remain constant once contact with a plant had been made, and all the Rothamsted solutions tended to level up to the reaction of the most alkaline, viz. pH 6·3. The speed at which this took place was correlated with the growth rate, being greater the better the seasonal conditions (Table 1), but in

TABLE 1. *Changes in* pH *value of three variants of the Rothamsted nutrient solution during growth*

(Samples bulked from ten plants.)

		Lettuce (Tom Thumb)						Red clover (S. 151)			
Age of plants ...		27 days		28 days		44 days		128 days		232 days	
Nutrient solution		March 1946		May–June 1946		Oct–Nov. 1946		January 1947		April–May 1947	
Approx. pH	Calcium (g./l.)	Initial pH	After 7 days	Initial pH	After 8 days	Initial pH	After 14 days	Initial pH	After 12 days	Initial pH	After 15 days
4·4	0·015	4·2	5·3	4·1	6·0	—	—	—	—	4·1	7·2
	0·030	4·2	5·4	4·1	6·5	4·3	4·9	4·3	5·8	—	—
	0·118	4·2	5·4	4·0	6·6	4·1	4·9	4·4	5·7	4·1	6·9
5·4	0·015	5·4	5·8	5·3	5·8	—	—	—	—	5·4	7·2
	0·030	5·4	5·8	5·3	6·3	5·5	5·7	5·3	6·0	—	—
	0·118	5·4	5·8	5·3	6·4	5·3	5·7	5·3	5·9	5·3	6·9
6·3	0·015	6·3	6·4	6·3	6·5	—	—	—	—	6·3	7·2
	0·030	6·3	6·4	6·3	6·5	6·1	6·3	6·3	6·3	—	—
	0·118	6·3	6·3	6·2	6·4	6·1	6·1	6·3	6·3	6·2	7·1

spite of the relatively short time during which the plants were subjected to the initial differences in pH value, the effect on growth was considerable. In the seedling stage the reaction of the medium was a particularly important factor, the most acid solution (pH 4·4) being definitely harmful, though the injury was largely mitigated if the calcium supply was adequate. At pH 5·4, no damage occurred and the plants closely resembled those in the solution at pH 6·3 where the best start was made, and

in neither case was the calcium supply of importance at this stage. The situation, however, changed rapidly, and after about 3 weeks the plants in the most acid solution were the best, provided the calcium supply was adequate, whereas at pH 6·3, all were uniformly poor irrespective of the calcium level. Growth in the solution at pH 5·4 was intermediate (Pl. 16, figs. 1–3). Confirmation of these results was shown by the final yields (Table 2), the effect being more marked with the Early

TABLE 2. *Effect of* pH *value and calcium supply on total dry weight in the presence and absence of molybdenum*

(g. per plant. Mean of five.)

Nutrient solution			Lettuce				Red clover	
			Cheshunt Early Giant		Tom Thumb		Early English	
	pH	Calcium (g./l.)	No Mo	With Mo	No Mo	With Mo	No Mo	With Mo
Rothamsted	4·4	0·015	2·80	4·88	4·10	6·27	11·39	19·23
		0·030	3·52	6·13	3·79	5·32	17·75	34·10
		0·118	3·73	6·53	4·41	6·22	15·64	46·40
Rothamsted	5·4	0·015	3·33	3·35	3·37	3·85	8·69	24·11
		0·030	2·64	4·30	2·91	4·46	12·19	32·79
		0·118	3·05	5·01	3·54	4·50	14·79	28·73
Rothamsted	6·3	0·015	2·11	2·03	2·46	3·02	6·67	26·12
		0·030	2·41	2·65	2·85	2·27	9·08	21·52
		0·118	2·47	1·96	2·38	2·32	15·53	30·18
Crone	6·4	0·213	4·50	7·48	3·92	6·17	18·24	37·52
S.E.			0·27		0·34		3·18	

Giant variety than with Tom Thumb. The greater importance of calcium for lettuce in solutions at pH 4 compared with pH 6 has also been described by Arnon & Johnson (1942). Specific calcium deficiency symptoms, such as curling and browning of the leaf margins, appeared first and were best defined in the most acid solution, though they were eventually recorded at all three pH values. Plants in the Crone solution (pH 6·4) were at first very similar in growth and appearance to those in the most alkaline Rothamsted medium (pH 6·3), but as they maintained their good condition, the eventual unsuitability of the Rothamsted solution must be ascribed to indirect effects rather than to its reaction.

Botrytis infection is common in lettuces grown under glass, and occurred in some of these experiments. Susceptibility to the disease was found to be closely associated with a low calcium supply, mortality being particularly high in the most acid solution where the demand for calcium was greatest (Table 3). Randomization of all treatments ruled out the possibility of infection being due to the position of the plants on the bench.

Response to molybdenum. The characteristic leaf lesions and pale colour of the shoots indicating a lack of molybdenum appeared after about 6 weeks from sowing Early Giant proving more sensitive than Tom Thumb. The deficiency symptoms

showed first in the plants in the most acid solution with the lowest calcium level, followed about a week later by all those in the two more acid media. In the most alkaline solution, the deficiency symptoms were further delayed, and in one instance failed to appear altogether. This latter set were carried on to the bolting stage, as earlier work (Warington, 1945) had indicated some influence of molybdenum in retarding the onset of this phase of growth. Delay in bolting, however, only reached significance in the more acid Rothamsted and in the Crone solution where molybdenum deficiency symptoms had already been recorded (Table 4).

TABLE 3. *Association between calcium supply and* Botrytis *infection in lettuce* (*Tom Thumb*)

Nutrient solution			
pH value	Calcium (g./l.)	Number of plants	Deaths
4·4	0·015	10	10
	0·030	10	6
	0·118	10	0
5·4	0·015	10	7
	0·030	10	1
	0·118	10	0
6·3	0·015	10	6
	0·030	10	2
	0·118	10	0

TABLE 4. *Lettuce* (*Tom Thumb*). *Effect of molybdenum on bolting* (*mean of five plants*)

	Shoot height (cm.)	
Nutrient solution	No Mo	With Mo
Rothamsted pH 4·4	29	17
Rothamsted pH 5·4	25	16
Rothamsted pH 6·3	12	12
Crone pH 6·4	15	11
S.E.	1·15	

Well-marked improvement in yield from the addition of molybdenum occurred in plants in all the Rothamsted solutions at pH 4·4, in half of those at pH 5·4, but in none of those at pH 6·3 (Table 2). However, the plants grown without molybdenum in the Crone solution, with a pH value approaching that of the most alkaline of the Rothamsted group, showed good visual symptoms of deficiency as well as a reduction in dry weight, so that the reaction of the medium alone could not account for the difference in behaviour of the plants in the variants of the Rothamsted solution. The conclusion to be drawn appears rather to be, that molybdenum deficiency symptoms are not limited to any special conditions of reaction or calcium supply in the culture solution (Pl. 16, fig. 4), but are likely to be most pronounced where growth is most

vigorous (Pl. 16, fig. 5). Walker (1948) has also found a correlation between the rate of growth and the need of lettuce for molybdenum. Benefit from molybdenum was not associated with the quantity of the element absorbed, for in another experiment with Tom Thumb, the only plants to show a significant increase in yield from the addition of sodium molybdate (those grown in the two most acid solutions) had the lowest molybdenum content in their shoots (Table 5). Although only a few figures are available, it appeared that where molybdenum was supplied its uptake tended to rise as the acidity of the nutrient solution was reduced just as has been found with soil (Stephens & Oertel, 1943). The molybdenum content of the control plants, on the other hand, was small and independent of the reaction of the medium, which suggests that what was present had been introduced with the seed rather than from impurities in the solution.

TABLE 5. *Total dry weight and molybdenum content of shoot of lettuce grown in solutions of different* pH *value and calcium content with and without molybdenum (mean of five plants)*

Nutrient solution			Total dry weight (g.)		Molybdenum content of shoot: p.p.m. of dry matter		Molybdenum content of shoot: γ per plant	
	pH	Calcium (g./l.)	No Mo	With Mo	No Mo	With Mo	No Mo	With Mo
Rothamsted	4·4	0·118	6·96	8·05	0·03	0·20	0·16	1·21
Rothamsted	5·4	0·030	3·30	5·11	—	0·43	—	1·66
		0·118	5·29	6·47	0·03	0·33	0·12	1·61
Rothamsted	6·3	0·030	3·18	4·29	0·04	0·69	0·09	2·22
		0·118	3·91	4·35	—	0·45	—	1·43
Crone	6·4	0·213	7·03	6·48	0·03	0·50	0·15	2·35
	S.E.		0·29					

Molybdenum deficiency increased the percentage total nitrogen content of the shoots, the effect being most marked where the highest rate of calcium was supplied (complete nutrients, Table 9).

Red clover

Two experiments, on similar lines to those with lettuce, were started in May and September 1946, and each carried on for 12 months. Garton's Early English and Aberystwyth S. 151 seed was used respectively. Nitrogen was supplied as potassium nitrate. Half of the autumn series were inoculated with *Rhizobium*, but the spring series were not inoculated. The nutrient solutions and their calcium content were the same as those described for lettuce, except that in the September set only two instead of three levels of calcium were tried (0·118 and 0·015 g. Ca/l.).

Results of treatment in the presence of molybdenum. As with lettuce, in the early stages of growth the importance of the calcium level in the Rothamsted type of solution varied with the reaction of the medium. A shortage of calcium was most

serious at pH 4·4, of little importance at pH 5·4 and even beneficial for a time at pH 6·3. These observations were borne out in the final yields of the plants, provided molybdenum was not lacking (Table 2), and confirmed in a subsequent experiment with wild white clover. The most acid solution produced the highest dry weight, but since the Crone nutrient (pH 6·4) gave nearly as good results, the favourable effect of the acidity was probably due to some indirect factor, such as improved availability of iron.

The pH changes in the solutions during growth closely resembled those described for lettuce, though when the clover was large, the reaction stabilized near the neutral point rather than at pH 6·3, supporting the findings of Hoagland (1919) (Table 1). Later data showed that this also occurred with well-grown lettuce (Text-fig. 2).

Response to molybdenum. Molybdenum deficiency in spring-sown clover showed best after the flowering culms had been cut back. The shoots became yellow-green, and the leaf margins rolled in on the upper surface, turning bluish and later light brown in colour (Pl. 17, figs. 10, 11). In the Rothamsted group of solutions, acidity apparently hastened the development of these symptoms, whereas the level of calcium was of little or no importance in this respect, as Walker (1948) had found in soil. Signs of molybdenum deficiency eventually appeared in all solutions, reaching a maximum in November, and as with lettuce, were best defined where growing conditions were most favourable. Visual symptoms then gradually disappeared but returned to an even more marked degree in the following spring (Pl. 16, fig. 7), suggesting that a need for molybdenum was associated with a phase of vigorous growth. A certain loss of plants is inevitable over a long-term experiment, but mortality was noticeably increased if molybdenum was lacking. Of fifty possible plants, only twenty-six were alive at the final harvest in the absence of molybdenum, compared with forty-six where the element was supplied. The final dry weights accordingly showed a large experimental error, but nevertheless a significant response to molybdenum was obtained in all except one treatment (Table 2).

In the autumn-sown experiment, response to molybdenum appeared simultaneously in the inoculated and uninoculated plants, though for a time they were best defined in the former series. An attack of red spider unfortunately vitiated much of the later results, but the main conclusions reached supported those previously described, viz. that in the absence of molybdenum, deficiency symptoms would develop in plants irrespective of the variations made in calcium content or pH value of the solutions in which they were grown.

(B) pH *value modified but calcium supply unchanged*

None of the cultures previously used had been alkaline in reaction. Plants were, therefore, grown in a number of variants of the Rothamsted and Crone solutions, containing the standard complement of calcium (0·118 g. Ca/l.), but with the pH value modified by hydrochloric acid or sodium hydroxide to cover a range of pH

from 4·2 to 8·2.* Each type of culture solution was made up in bulk unmodified. pH adjustments were carried out immediately before use on a volume sufficient for five or usually ten plants (4–6 l.), so that the medium for the comparable replicates, grown with and without molybdenum should be identical. Variation in pH value between different batches of solution did not usually exceed 0·2, and in view of the rapid changes in reaction known to occur after contact with the plant, a similar latitude was allowed when adjusting the solutions. On several occasions when the cultures were being renewed, the pH of the solutions in which plants had been grown was determined, for though nutrient solutions supplying nitrogen as nitrate normally become more alkaline after contact with the plant, little was known as to their behaviour when their reaction had been modified with hydrochloric acid or sodium hydroxide. Preliminary tests had shown that under these circumstances slow changes in pH values take place even in the absence of the plant. Rothamsted and Crone solutions of pH 5·7 and 6·3 respectively, when modified to pH 4·3 and left standing in glass vessels, reverted to pH 5·7 and 5·9 in a fortnight, after which time they remained constant. A similar Rothamsted solution modified to pH 7·3, however, showed no alteration over the same period. Contact with the plant greatly increased the rate of change, and in the case of the alkaline solutions reversed its direction. Details of the changes brought about by lettuce and clover in the presence and absence of molybdenum are discussed below.

Lettuce

Four experiments were carried out with lettuce in 1947 and 1948, Cheshunt Early Giant being grown in the winter and Tom Thumb in the summer months.

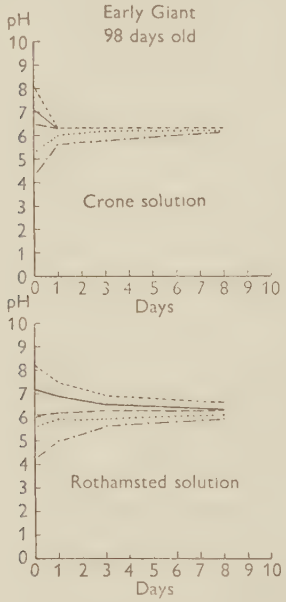
Results of treatment in the presence of molybdenum. In spite of the wide range of reaction covered (pH 4·2–8·2), all solutions soon levelled up to a pH of approximately 6–7. The change was rather more rapid in the Crone than in the Rothamsted series, but in both the rate depended on the age of the plant, being slowest in the young stages and sometimes slackening off again when the plants were past their prime. Large lettuces altered the reaction of the solution appreciably in 24 hr. and equilibrium was almost reached after 3 or 4 days, whereas young plants required more than 10 days to achieve the same result (Text-figs. 1 and 2).

As in the previous experiment, growth, particularly of the roots, received a temporary check in the most acid solution. Injury from the alkaline media, on the other hand, showed itself chiefly in the shoot and lasted till the plants were mature, reducing their size and giving the leaves a tough and speckled appearance. Damage of this type was only pronounced in the modified Rothamsted solution and was probably due to a lack of iron through precipitation, which would not occur with the Crone formula.

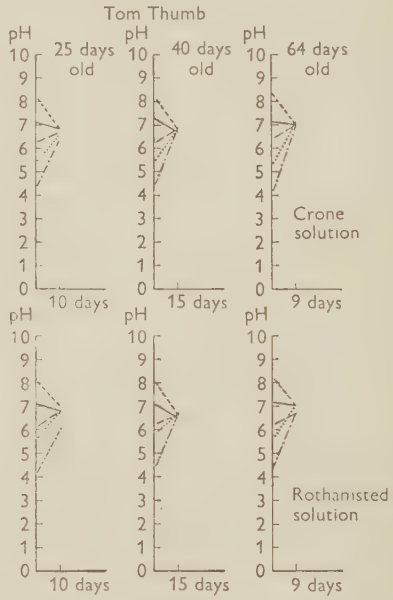
Response to molybdenum. Differences in dry weight due to molybdenum reached

* Mean pH of twenty-eight batches of solution. Rothamsted 4·2, **5·7**, 6·2, 7·2, 8·2. Crone 4·3, 5·3, **6·3**, 7·2, 8·2. Unmodified in black figures.

significance in plants grown in three of the Rothamsted solutions (pH 4·2, 6·2 and 7·2). Visual symptoms of molybdenum deficiency, however, appeared in the set in the unmodified solution (pH 5·7), so that some form of response occurred at all reactions except the most alkaline (pH 8·2). There was also a significant improvement in yield in the plants supplied with molybdenum in each of the Crone modifications, and though visual symptoms of deficiency were in general good (Pl. 16, fig. 6), they were missing in the two more acid and the most alkaline media. On the whole, therefore, response to molybdenum was independent of the reaction of the medium, with the possible exception of the solution at pH 8·2, where the results were rather conflicting and probably complicated by indirect factors.



Text-fig. 1.



Text-fig. 2.

Text-figs. 1, 2. pH changes in nutrient solutions during growth. Lettuce. (Mean of ten plants.)

The percentage total nitrogen content of the shoot was consistently higher where molybdenum was not added, the mean value for the two series being given in Table 9.

Red clover

Results of treatment in the presence of molybdenum. A single experiment, carried out in July 1947–8 with treatments identical to those described for lettuce, gave very similar results. The most acid solution (pH 4·3) checked early growth, particularly of the roots in both Crone and Rothamsted solutions, though good recovery took

place later. The most alkaline Rothamsted solution (pH 8·2) caused chlorosis and a reduction in size, but otherwise the reaction of the solution seemed to have little effect on growth (Table 6).

TABLE 6. *Total dry weight and molybdenum content of shoot of red clover grown in solutions of different* pH *value, with and without molybdenum (mean of five plants)*

Nutrient solution	pH	Total dry weight (g.)		Molybdenum content of shoot (p.p.m. dry matter)	
		No Mo	With Mo	No Mo	With Mo
Rothamsted	4·2	59	58	0·45	29·2
	5·7	54	62	0·20	28·7
	6·2	53	77	0·58	24·5
	7·2	53	63	0·45	23·5
	8·2	67	41	2·09	58·5
Crone	4·3	62	92	0·06	27·8
	5·3	61	65	0·18	26·5
	6·3	59	66	0·13	19·6
	7·2	51	75	0·35	28·3
	8·2	51	71	0·27	25·4
S.E.		7·1			

Response to molybdenum. Differences due to molybdenum did not show until the autumn. As on the previous occasion, the symptoms of deficiency disappeared during the winter but reappeared with greater intensity in the spring. Damage from azobenzene fumigation unfortunately caused a break in the records and much individual variation in dry weight, but well-defined visual symptoms developed in plants in all solutions except Rothamsted pH 8·2. (For the Crone series see Pl. 17, fig. 8.) A significant increase in yield due to molybdenum was obtained on the experiment as a whole and in solutions at pH 4·2, 6·2 and 7·2. It seemed, therefore, that with the possible exception of the most alkaline Rothamsted solution, which gave anomalous results both as regards dry weight and molybdenum content, the reaction of the medium had little influence on the response of the plant to molybdenum. Some casual infection with *Rhizobium* had occurred by the end of the experiment, nodules developing most freely on the plants lacking molybdenum, as Anderson & Thomas (1946) had found. The presence or absence of nodules, however, did not account for any observed differences in colour or size between replicates.

Where molybdenum was supplied, the quantity accumulated in the leafy part of the shoot during 12 months was considerable, and with one exception was of much the same order irrespective of the reaction of the medium or the type of culture solution used (Table 6). The plants grown without added molybdenum contained an appreciable though variable amount of the element in spite of having shown well-defined visual symptoms of molybdenum deficiency. The quantity present, however, did not account for the significance or otherwise of the differences in dry weight due to molybdenum, nor show any correlation with the pH value of the nutrient solution.

The exceptionally high molybdenum content of both sets of plants grown in the most alkaline Rothamsted solution must be regarded as anomalous, as no satisfactory explanation seems to be forthcoming to account for it.

EFFECT OF ALTERATIONS IN THE NITROGEN SUPPLY IN THE PRESENCE AND ABSENCE OF MOLYBDENUM

Molybdenum, as well as manganese, is generally regarded as essential for the assimilation of nitrate, and both the total and particularly the NO_3-nitrogen content of plants suffering from molybdenum deficiency is usually abnormally high (Mulder, 1948; Hewitt, Jones & Williams, 1949). Some indication of the plant's status in respect to molybdenum might, therefore, be expected from nitrogen analyses. Further, a possible relationship between the demand for molybdenum and the level of the nitrogen supply had been suggested by the occasional occurrence of leaf withering and rolling at the margins (indicative of molybdenum deficiency) in red clover grown in solutions containing 0·1 p.p.m. molybdenum but low in nitrogen (author, unpublished). Plants were accordingly set up in solutions providing different amounts of nitrogen and molybdenum and analyses made of the total and NO_3-nitrogen content in the shoots produced.

Lettuce

Five experiments were carried out during 1948 and 1949. The quantity of nitrogen normally supplied by the Rothamsted and Crone solutions (0·139 g. N/l.) was taken as standard, and plants set up in a range of solutions containing one-quarter to three times this amount. Each nitrogen treatment was repeated with the addition of 0·1 p.p.m. molybdenum, and a higher rate of 5 or 10 p.p.m. molybdenum included for the 1949 summer and winter lettuce experiments respectively. The plants were generally grown until hearted, but on two occasions were sampled at three different stages of growth.

Results of treatment in the presence of molybdenum. The effect of the nitrogen supply on growth was rather variable, but, in general, the quarter or half standard rates produced definitely nitrogen-deficient plants, while no further improvement occurred when the supply was increased beyond twice the standard amount. (The solutions were renewed fortnightly.)

Response to molybdenum. The degree of response to molybdenum varied in the different experiments, but benefit, either visual or as an increase in dry weight, was obtained on some occasions with all nitrogen treatments up to twice standard, the response being usually most marked in the older plants and where the nitrogen supply was adequate. For example, in one experiment increases in yield due to molybdenum occurred where standard or half standard nitrogen was supplied, but not if only the quarter rate were given, nor in the earliest sample (Table 7). A reduction in the percentage of both total and NO_3-nitrogen in the shoot accompanied these increases in yield and also occurred in the 7-week-old plants grown with

molybdenum at the two higher levels of nitrogen, though dry weight differences were only small at this stage. Where the lowest quantity of nitrogen was given, the total nitrogen content of the shoot was reduced by the presence of molybdenum, but no conclusions could be drawn regarding its effect on NO_3-nitrogen as the values were too small to be reliable.

TABLE 7. *Response of lettuce to molybdenum when grown in solutions containing different amounts of nitrogen. Lettuce* (*Tom Thumb*). (*Mean of five plants*)

	Total dry weight (g.)			% N in dry matter of shoot					
Days from sowing ...	44	63	80	44		63		80	
Treatment				Total N	NO_3-N	Total N	NO_3-N	Total N	NO_3-N
¼ Standard N									
No Mo	1·12	2·82	4·35	2·71	0·001	1·60	0·005	1·70	0·01
0·1 p.p.m. Mo	1·10	3·19	4·28	2·58	0·01	1·57	0·01	1·54	0·005
5·0 p.p.m. Mo	0·89	3·19	4·33	2·61	0·01	1·46	0·01	1·54	0·01
½ Standard N									
No Mo	1·02	3·11	4·36	2·99	0·05	2·31	0·02	2·32	0·07
0·1 p.p.m. Mo	1·08	3·68	5·53	3·37	0·03	1·95	0·002	1·98	0·01
5·0 p.p.m. Mo	0·95	3·08	5·21	3·49	0·05	1·99	0·01	1·98	0·01
Standard N									
No Mo	1·15	2·89	3·65	3·07	0·08	2·91	0·16	2·87	0·22
0·1 p.p.m. Mo	1·02	4·13	7·03	3·66	0·09	2·63	0·05	2·44	0·04
5·0 p.p.m. Mo	0·88	3·80	6·06	3·67	0·10	2·68	0·03	2·47	0·02
S.E.			0·31						

Standard N = 0·139 g. N/l.

TABLE 8. *Effect of nitrogen and molybdenum supply on dry weight and nitrogen and molybdenum content of shoot. Lettuce* (*Cheshunt Early Giant*). *Mean of five plants*)

Treatment	Dry weight (g.)	p.p.m. dry matter Mo	% dry matter	
			Total N	NO_3-N
¼ Standard N, no Mo	2·60	0·17	1·74	< 0·01
0·1 p.p.m. Mo	2·81	0·60	1·56	< 0·01
10·0 p.p.m. Mo	2·57	65·0	1·64	< 0·01
½ Standard N, no Mo	3·72	0·18	1·83	0·02
0·1 p.p.m. Mo	3·78	0·95	1·76	< 0·01
10·0 p.p.m. Mo	3·20	64·8	2·13	0·01
Standard N, no Mo	4·09	0·26	2·55	0·15
0·1 p.p.m. Mo	4·44	0·90	2·21	0·03
10·0 p.p.m. Mo	3·29	73·4	2·41	0·04

(0·139 g. N/l.)

A similar influence of molybdenum on nitrogen content was found in a set of plants which had failed to show any benefit from the element as regards dry-weight production (Table 8). Further, the mean* total-nitrogen content of lettuce grown

* Values for plants grown in unsuitable solutions, e.g. those with calcium deficiency, molybdenum excess or pH 8·2, have been omitted from the mean.

under a variety of conditions in nine different experiments was significantly lower where molybdenum was added (Table 9). Comparable figures for the NO_3-nitrogen content are unfortunately not available for this series. It is, however, clear that although the addition of molybdenum increased both dry-matter production and the amount of nitrate reduced, a considerable proportion of the nitrogen absorbed had been converted into the organic form whether or not molybdenum were supplied.

TABLE 9. *Mean percentage total nitrogen in dry matter of shoot of mature lettuce grown with and without molybdenum*

Treatment*		No. of plants	No Mo	0·1 p.p.m. Mo
Complete nutrients	1946	5	3·86	2·72
pH range 4·2–7·2	1947–8	20	3·56	2·95
pH range 4·2–7·2 Crone solution		20	3·50	2·55
N varied ($\frac{1}{2}$ to 2) × Standard	1948	25	2·28	2·02
N varied ($\frac{1}{2}$ to 2) Crone solution		25	2·50	2·10
N varied ($\frac{1}{4}$ to 3) × Standard	1948	25	3·21	2·93
N varied ($\frac{1}{4}$ to 3) Crone solution		25	3·09	3·06
N varied ($\frac{1}{4}$ to 1) × Standard	1949	15	2·04	1·84
N varied ($\frac{1}{4}$ to 1)		15	2·30	1·98
		Mean	2·93	2·46
		Difference	0·47 ±	0·136

* Rothamsted solution unless otherwise stated.

The higher rates of molybdenum proved slightly toxic when the plants were small, causing chlorosis and checking growth. There were some indications that liability to damage was greatest where most nitrogen was supplied, and this received support from the slightly increased uptake of molybdenum by the plants receiving such treatment (Table 8). On the other hand, there was no evidence that an increase in the supply of molybdenum was of any benefit when nitrogen was deficient.

Red clover

Two experiments were carried out with clover, the same quantity of nitrogen in the nutrient solution being taken for standard as had been used for lettuce (0·139 g. N/l.). In 1948, levels of half standard and twice standard nitrogen were compared in both Rothamsted and Crone solutions, each with and without inoculation, and in the presence and absence of 0·2 p.p.m. molybdenum. In the following year, Rothamsted solution only was used, without inoculation, and the nitrogen supply reduced to one-quarter, one-half and standard respectively. Molybdenum treatments consisted of 0·1 p.p.m. or 5 p.p.m. molybdenum in this case. Samples were taken at two stages of growth. For approximately 1 month from sowing all seedlings received half standard nitrogen to allow of better initial selection for uniformity, and to encourage nodulation where *Rhizobium* was added.

Results of treatment in the presence of molybdenum. Differences in growth due to nitrogen supply began to show after about 1 month and, as would be expected, were

most marked in the uninoculated set. Significant increases in dry weight were obtained with each increment of nitrogen, but the percentage total or NO_3-nitrogen content of the shoot failed to show any correlation with the nitrogen supplied.

Response to molybdenum. In the 1948 experiment, good symptoms of molybdenum deficiency developed in the autumn. They showed first in the plants receiving least nitrogen, whether inoculated or not, and failed to appear in the set supplied with the twice standard rate. By December, when the uninoculated plants were harvested, the visual symptoms had largely disappeared and no significant reduction in dry weight nor increase in percentage total nitrogen content of the shoot in the absence of molybdenum was obtained at any nitrogen level. To ensure conditions of nitrogen deficiency in the inoculated plants which were carried on, the nitrogen supplied in the half standard solution was then reduced to one-quarter and in March omitted altogether. With the onset of vigorous growth in the spring, signs of molybdenum deficiency reappeared, this time showing at all levels of nitrogen (Pl. 17, fig. 9). Paleness and reduction in size were universal symptoms, but marginal leaf rolling seemed to be specially associated with conditions of low nitrogen supply and was again noticed on a few plants provided with molybdenum. As before, nodules were much more plentiful on the plants lacking molybdenum. In contrast to lettuce, therefore, a reduction in nitrogen supply helped to accentuate the effects of molybdenum deficiency to some extent, though in both crops symptoms were ultimately best defined when growth was most vigorous.

A significant increase in dry weight due to molybdenum was obtained on the experiment as a whole, though the individual differences were better defined in the Rothamsted than in the Crone series, reaching significance at all levels of nitrogen in the former case (Table 10). Differences in the NO_3-nitrogen content of the shoot,

TABLE 10. *Response of red clover to molybdenum when grown in Rothamsted solutions containing different amounts of nitrogen.* (*Mean of five plants*)

Nitrogen treatment (all inoculated)		Total dry weight (g.)
$\frac{1}{2}$N→ON	No Mo	40·7
	0·2 p.p.m. Mo	67·8
1 N	No Mo	73·6
	0·2 p.p.m. Mo	109·5
2 N	No Mo	109·0
	0·2 p.p.m. Mo	138·6
(N = 0·139 g. N/l.)		S.E. 6·51

due to molybdenum treatment, were only marked where the largest quantity of nitrogen was supplied. Here the percentage in the dry matter was 0·13 and 0·065 for the plants grown without molybdenum in the Rothamsted and Crone solutions respectively, compared with 0·005 and 0·01 where molybdenum was given. Plants grown at the lower levels of nitrogen gave figures too small to be reliable.

Little further information was obtained in 1949. The higher rate of 5 p.p.m. molybdenum proved toxic to young plants at all levels of nitrogen, though recovery took place rather sooner where the nitrogen supply was low, confirming the results with lettuce. A few of the nitrogen-deficient plants showed marginal leaf curling, and though the experimental conditions were admittedly limited, there was no evidence that the higher rate of molybdenum was of any value in overcoming this symptom. Its occurrence in the presence of molybdenum could be explained if it resulted from the lack of some process or substance within the plant, for which both adequate nitrogen and molybdenum were required.

DISCUSSION

Although changes in calcium content and/or pH value of the nutrient solution affected the growth of lettuce and red clover very similarly and did not alter the need of either plant for molybdenum, variation in the amount of nitrogen supplied influenced the two crops somewhat differently. With clover, the appearance of molybdenum deficiency symptoms was hastened when nitrogen was scarce, whereas with lettuce it was retarded. Since both inoculated and uninoculated clover behaved alike in this respect, the different result with lettuce could not be attributed to its being a non-legume, though molybdenum is known to be essential for symbiotic fixation of nitrogen. The explanation probably lies in the different molybdenum requirement of the two plants. The demand for molybdenum varies considerably with different species, and also appears to be influenced by the rate of growth. A quantity of the element (possibly available from the seed) might, therefore, be sufficient for one species under conditions of nitrogen deficiency when growth would be slow, which would not suffice under better growing conditions or for another species with a larger molybdenum demand. A higher molybdenum requirement on the part of red clover compared with lettuce could thus explain the difference in the response of the two crops to the element with respect to the nitrogen supply.

My best thanks are due to Dr R. L. Mitchell and Mr H. H. Le Riche for spectrographic analyses of plant material, to Dr P. S. Nutman for cultures of *Rhizobium* and to Miss Margery Andrews for the nitrogen and statistical analyses.

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Fig. 1

Fig. 2

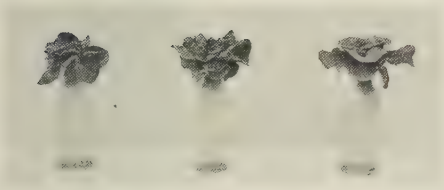

Fig. 3

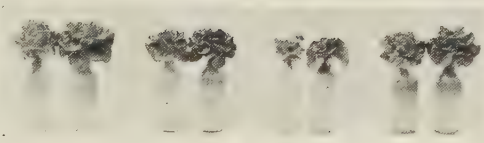

Fig. 4

Fig. 5

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Fig. 7

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Fig. 8

Fig. 9

Fig. 11

Fig. 10

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EXPLANATION OF PLATES 16 & 17

(Photographs by V. Stansfield)

Figs. 1–3. Effect of calcium supply on lettuce grown in solutions of pH 4·4, 5·4 and 6·3 respectively. L–R, $\frac{1}{8}$, $\frac{1}{4}$, standard calcium (0·118 g./l.). All with molybdenum.

Fig. 4. Response of lettuce to molybdenum when grown in solutions of different pH. Standard calcium throughout. L–R, Rothamsted solutions pH 4·4, 5·4, 6·3, Crone solution pH 6·4. Each pair: no molybdenum on left, 0·1 p.p.m. molybdenum on right.

Fig. 5. Response of lettuce to molybdenum when grown in Rothamsted solutions pH 4·4 with different calcium contents. L–R, $\frac{1}{8}$, $\frac{1}{4}$, standard calcium (0·118 g./l.). Each pair: no molybdenum on left, 0·1 p.p.m. molybdenum on right.

Fig. 6. Lettuce. L, showing molybdenum deficiency symptoms. R, healthy plant, 0·1 p.p.m. molybdenum supplied.

Fig. 7. Response of red clover to molybdenum when grown in solutions of different pH. Standard calcium throughout. L–R, Rothamsted solutions pH 4·4, 5·4, 6·3, Crone solution pH 6·4. Each pair: no molybdenum on left, 0·1 p.p.m. molybdenum on right.

Fig. 8. Response of red clover to molybdenum when grown in Crone solutions of different pH. L–R, pH 4·3, 5·3, 6·3, 7·2, 8·2. Each pair: no molybdenum on left, 0·2 p.p.m. molybdenum on right.

Fig. 9. Response of red clover to molybdenum when grown in Crone solutions of different nitrogen content. L–R, $\frac{1}{2}$, 1, $\times 2$ standard nitrogen (0·139 g./l.).

Figs. 10, 11. Red clover showing molybdenum deficiency symptoms on leaves.

(*Received* 4 *April* 1950)

THE PHYTOTOXIC EFFECTS OF D.D.T., B.H.C., PARATHION AND TOXAPHENE ON TOBACCO

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(With 11 Text-figures)

No phytotoxic effect was seen following a pre-sowing spray of tobacco seed-beds with 27 lb./acre technical D.D.T. or after an application of the same material at 75·6 lb./acre to 3-week-old tobacco seedlings.

A pre-sowing application of parathion (diethyl *para*nitrophenyl thiophosphate) (2% dust) at 1·8 lb. parathion per acre had no harmful effect. Used on 3-week-old tobacco seedlings at the excessive rate of 22·7 lb./acre it caused serious stunting and many deaths.

Toxaphene (chlorinated camphene: empirical formula $C_{10}H_{10}Cl_8$), applied as a 25% wettable powder in a pre-sowing spray at 6·4 lb. toxaphene per acre, did not injure tobacco seedlings.

No residual phytotoxic effects appeared in beds re-sown 4 months after being treated with parathion or toxaphene at the pre-sowing doses given above.

Benzene hexachloride, applied before sowing at doses above 1·6 lb. technical B.H.C. per acre, suppressed root development in newly germinated tobacco seedlings. B.H.C. dusts used on 11-day-old seedlings at 2·25 lb. technical B.H.C. per acre caused temporary distortion and stunting. Up to 11 lb./acre these symptoms were transitory: at 37·5 lb./acre many plants were killed and the remainder severely stunted. Resistance to these phytotoxic effects increased with age of plant, but 3-week-old tobacco seedlings showed considerable mortality after the application of 75·6 lb./acre of technical B.H.C.

Beds re-sown 4 months after the application of 6·4 and 12·8 lb. respectively of technical B.H.C. per acre showed no phytotoxic effect, but, as tobacco seed is sown on the soil surface, the effect of the B.H.C. may have been merely masked, and it is not safe to assume that there was no residual effect. The actual persistence of B.H.C. in the soil was not determined.

The possible mechanism of action of the B.H.C. effect is discussed.

Since the introduction of D.D.T. as an insecticide, followed by B.H.C., parathion, and toxaphene, a great deal of work has been done on the insecticidal evaluation of these chemicals. Comparatively little has been published on their phytotoxic effects. In most cases, the chemicals have been found safe on a wide variety of plants, but phytotoxicity is known to be associated with B.H.C. in particular (Stoker, 1948; Cullinan, 1949; Stitt & Evanson, 1949; Dogget & Lilly, 1949).

With the tobacco crop, special care is required in the application of insecticides. Tobacco is subject to insect attack from its earliest stages, and in commercial seed-bed culture insecticide application is usually a necessity. In addition, when considering the application of chemicals to field stand tobacco, nothing can be used which has any deleterious effect on the quality of the leaf.

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The work described in this paper aims at establishing some standards of safety for the use of the above-named insecticides on the tobacco crop, in Southern Rhodesia. Special attention has been paid to the seedling plants, which are particularly susceptible to damage.

The effect of B.H.C. in laboratory trials

Early in 1948, Petri-dish germination tests were carried out with Virginia tobacco. The seed had been dusted with B.H.C. preparations of 3 and 50% technical B.H.C. content. The seed was mixed in a tube with about half its volume of dust and stored at room temperature in contact with the B.H.C. Kaolin was mixed with control seeds, as this was the diluent of the active dusts. A visible amount of dust adhered to the seeds (excess being blown off) when they were set up for germination on wet filter-papers in Petri dishes. The criterion of germination was when a seedling had the two cotyledonary leaves withdrawn from the testa.

A typical result is shown in Fig. 1: the effect of the B.H.C. is apparent.

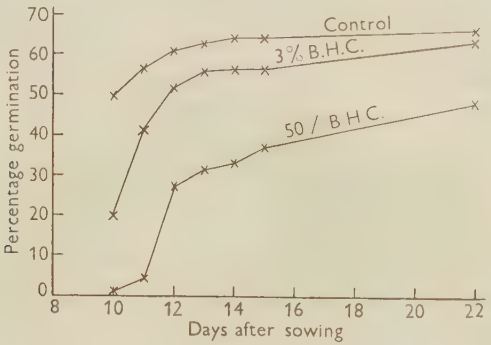

Fig. 1

The gross morphological effects of the B.H.C. on the seedlings are shown in Fig. 2. B.H.C. appears to have suppressed the development of the radicle, and B.H.C.-treated seedlings show a reduction in the number of root hairs, this being almost complete in the case of the 50% B.H.C. dust. Smith (1948*b*) observed similar phenomena on cress seedlings.

The above results were obtained with freshly treated seed, but repetition of germination tests on three subsequent occasions over a period of several months showed substantially the same effect, and not an increasing effect of the B.H.C. with the time of storage. The hypocotyl of the worst-affected seedlings displays a swelling, consisting of cells of greater than normal size, suggesting that the B.H.C. has some effect on cell-division. For these tests, local seed harvested in 1947 was used.

The effect of parathion as a seed treatment

Tobacco seeds were moistened with a parathion suspension containing 37·5 p.p.m. of active parathion (equivalent to $\frac{1}{4}$ lb. of 15% wettable powder per 100 gallons). Germination tests showed no significant effect from the treatment.

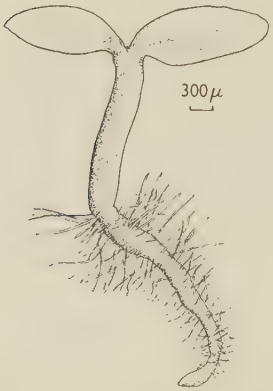

Fig. 2*a*. Control seedling.

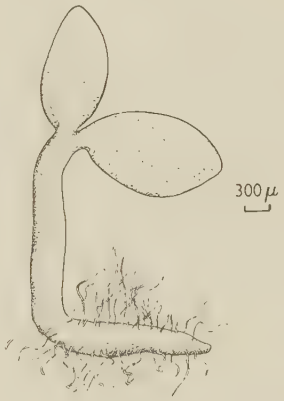

Fig. 2*b*. 3% B.H.C. seedling.

Fig. 2*c*. 50% B.H.C. seedling.

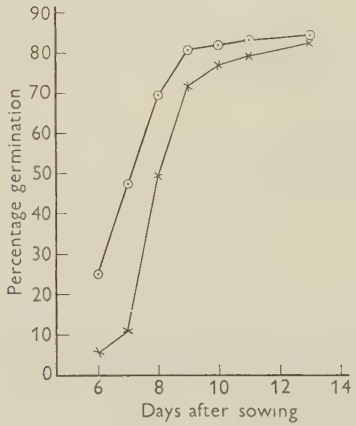

Fig. 3. Petri dish test: 20. i. 49.

×——× Parathion seeds. ⊙——⊙ Control seeds.

This was repeated with tobacco seed dusted with 15% parathion wettable powder in the manner described above for the B.H.C. tests. The germination results are shown in Fig. 3, which gives the mean results for three treated and three control dishes. A total of 2800 seeds was used in this experiment.

There appears to have been a slight depressant effect on germination whereas Dogget & Lilly (1949), found that parathion accelerated growth and germination in corn), but no morphological effects of the parathion could be detected, and at normal dosages (e.g. as applied to the soil) it seemed unlikely that any harm would result from the contamination of seeds, or the treatment of seed-beds, with parathion.

Trials with D.D.T. applied directly to tobacco seed-beds

No laboratory tests were made on the effect of D.D.T. on germination, but a field trial was carried out at Avondale, Salisbury, Southern Rhodesia, on fairly heavy soil, in the 1947–8 season. Eight seed-beds, each 1/726 of an acre in extent, were used: four were 'burnt' and four left unburnt. In commercial practice burning is done to lessen the weed growth on the seed-beds, and it also assists by increasing the potash content, and raises the pH of the surface soil considerably.

Burning was done by firing a 2 ft. deep layer of dry grass and dry maize trash on the beds. After the fire had burnt out, all larger fragments of charred material were raked off, and the finer ash was worked into the surface soil.

Fertilizer was then broadcast on to the beds at the rate of 10 lb./100 sq.yd. (Taylor, 1927) and raked in. Commercial grade fertilizer was used, rated at 13 (P_2O_5), 6 (N_2), 8 (K_2O). The D.D.T. was applied next (on 17 November 1947) in the form of a 50% D.D.T. wettable powder preparation, as water suspensions of 0·5, 1·0 and 2% D.D.T., at 136 gallons spray per acre. This gives dosages of 6·8, 13·6 and 27·2 lb. of technical D.D.T. per acre respectively. Spraying was done with a pressure type knapsack sprayer operating at 25–30 lb./sq.in. This gave a spray which did not disturb or wash away the surface soil, and allowed the insecticide to be deposited evenly on the soil surface.

As soon as the spray had dried, the beds were sown to Virginia tobacco (variety Jamaica Wrapper) at the rate of 1 level teaspoonful per 10 sq.yd. (Taylor, 1927). The seed was mixed with dry soil and broadcast, then watered in. The beds were covered with grass screens, which were raised up as the plants grew, and the beds were freely watered at regular intervals.

Nine days after sowing, germination commenced. A good germination resulted and an even stand was obtained on all eight beds. The eight beds comprised four burnt, and four unburnt, and in each of these two series there was one control bed and one bed sprayed at each of the three D.D.T. dosages. No differences were noticeable between the beds at this date. Twelve days after sowing, considerably less weed growth distinguished the burnt beds, but otherwise no differences were apparent. Twenty-eight days after sowing a difference in vigour of plants between the burnt and unburnt beds had developed. Thirty-three days after sowing, the highest D.D.T.-dosed beds had a slightly inferior growth, though the stand of plants was normal, compared with all other beds. Forty-three days after sowing, the plants then being about 9 in. tall, the lowest D.D.T.-dosed beds (6·8 lb./acre) were the best, but the differences between the burnt and unburnt beds were greater than

the differences ascribable to the D.D.T. From the commercial aspect, all the beds would have been considered satisfactory. The beds were then left for the plants to grow away, and 85 days after sowing, all differences between the beds had been submerged. The plants were then 18–24 in. tall, growing vigorously, and normal in all respects.

It appears from this experiment, that no great danger is to be expected from the use of D.D.T. on tobacco seed-beds as a pre-sowing treatment. The dosages tested were in excess of those normally employed for control of insects on the seed-beds.

Trials with B.H.C., D.D.T. and parathion at excessive doses

The tobacco selected for this experiment was a bed of Turkish seedlings sown on 10 February 1948, on the same site as the previous trials. The soil had received no previous insecticidal treatment at any time. Growth and germination were very even, and the stand of plants was uniform in density and height of plant cover, when the treatments were applied.

Twenty-eight days after sowing, the following treatments were applied to 1 sq.yd. sections of the seed-bed, each treatment being next to a control square. A 50% D.D.T. wettable powder, a 50% B.H.C. (5% γ-isomer) wettable powder, and a 15% parathion wettable powder were applied so as to give the following quantities per acre:

D.D.T. technical	75·6 lb.;
B.H.C. technical	75·6 lb.;
Parathion	22·7 lb. active ingredient.

The powders were broadcast evenly over each plot. Sixty-four hours later, the D.D.T. and B.H.C. plots were normal, but on the parathion plot a high percentage of the plants were scorched, and a few days later heavy scorch and the death of many seedlings occurred on the B.H.C. plot.

About 3 weeks later, the B.H.C. section showed the poorest growth, and the parathion-treated plants had recovered somewhat and showed fair growth. The D.D.T.-treated plot showed excellent growth in contrast. The average height of the plant cover on all plots was measured 27 days after treatment, and is shown in the histogram of Fig. 4. The two control plots adjacent to the B.H.C. and parathion plots showed marked depression of growth, due it is thought to redistribution of the insecticides by heavy rains.

In addition, a number of plants were taken from each treated plot, and from the check plot adjoining the D.D.T. section. Care was taken to disturb the root system as little as possible. The plants were washed free of soil, and the maximum root length and the distance from the ground-level to the tip of the longest leaf were measured to the nearest $\frac{1}{4}$ in. These figures are shown plotted in Fig. 4, each point representing the measurements of one plant. The straight lines are drawn by eye.

Eighty-seven days after treatment, no differences could be seen between the

D.D.T. plot and the adjoining control. The plants on the other four plots were still much smaller, but were growing quite well.

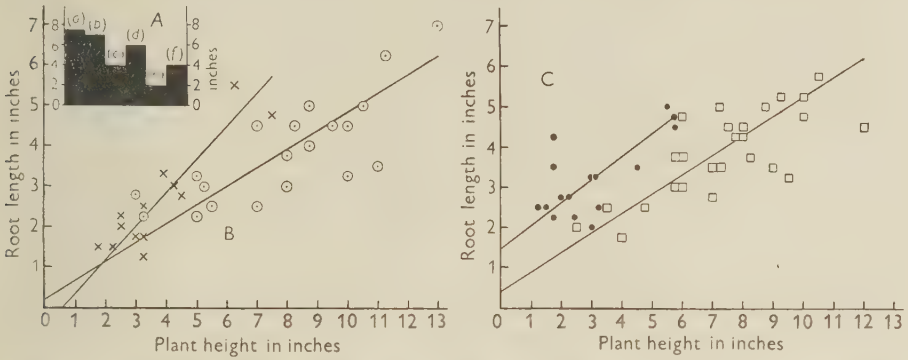

Fig. 4. A, ordinate values = average plant height on each plot: (*a*) D.D.T., (*b*) control, (*c*) parathion, (*d*) control, (*e*) B.H.C., (*f*) control. B, × = parathion, ⊙ = control next to D.D.T. C, ● = B.H.C., □ = D.D.T.

Pre-sowing treatment of tobacco seed-beds with B.H.C., toxaphene, and parathion preparations, each with D.D.T.

Pre-sowing treatments were applied to a series of tobacco seed-beds in the form of low-pressure sprays, as described above. All the beds were 1/363 of an acre, and 1 l. of spray was applied per bed. On all beds except the control (*F*) D.D.T. wettable powder was included in the spray at a dose of 8·00 lb. technical D.D.T. per acre.

Table 1. *Schedule of applications*

Bed	Chemical	Concentration/l. (g.)	Rate/acre
A	B.H.C. 50% wettable powder	2	0·80 lb. technical B.H.C.
B	B.H.C. 50% wettable powder	4	1·60 lb. technical B.H.C.
C	Parathion 15% wettable powder	2	0·24 lb. active ingredient
D	Toxaphene 25% wettable powder	4	0·8 lb. toxaphene
E	Toxaphene 25% wettable powder	8	1·6 lb. toxaphene
F	Control	—	—

The rates of application of the other insecticides are given in Table 1. The seeding rate and fertilizer application were exactly as described in the first seed-bed tests with D.D.T. above, but in this case none of the beds was burnt before sowing. The tobacco sown was Virginia (variety Bonanza).

Germination began on the sixth day after sowing and, commencing then, random counts in 6 in. square quadrats were made of the number of seedlings that had appeared. Twelve counts were made on each bed at each sampling date, and fourteen sets of records were obtained up to 34 days after sowing, when counts were stopped.

The figures obtained showed no significant differences between beds *A*, *B*, *D* and *E*, which gave counts on the 34th day of $42{\cdot}42 \pm 4{\cdot}50$, $38{\cdot}00 \pm 5{\cdot}74$, $39{\cdot}48 \pm 3{\cdot}94$ and $36{\cdot}00 \pm 4{\cdot}48$ respectively. Beds *C* and *F* were consistently much lower, giving a 34-day count of $29{\cdot}42 \pm 3{\cdot}83$ and $12{\cdot}33 \pm 2{\cdot}39$ respectively. (The figures are the numbers of seedlings counted per 36 sq.in.)

Since *F*, the control bed, gave such low counts it is of little value for purposes of comparison. In actual fact, the subsequent stand and growth of plants on beds *A* to *E* was very good. Under the conditions of the experiment, the treatments applied were not harmful to the tobacco, and safe to use in practice. The lower average of beds *C* and *F* is difficult to explain, but may have been due to soil differences, or to a lack of protection from insect pests on these beds.

To confirm the safe nature of D.D.T. on tobacco seed-beds, a further experiment was laid out, and counts made as before. D.D.T. was applied at 8·00 lb. of technical D.D.T. per acre, as a 50% wettable powder suspension. Four half-beds were used, and after 22 days the counts were as follows:

Bed 1	D.D.T. $23{\cdot}92 \pm 6{\cdot}05$	Control $26{\cdot}83 \pm 5{\cdot}43$
Bed 2	D.D.T. $13{\cdot}92 \pm 4{\cdot}42$	Control $14{\cdot}00 \pm 3{\cdot}49$

There is no significant difference due to the D.D.T. application, but the difference in germination between beds is very large, thus agreeing with the previous results.

Further trials with B.H.C. applied to beds before sowing

Since the Petri dish tests of germination showed that B.H.C. was capable of producing harmful effects on tobacco seedlings, an experiment was designed to show if these could be reproduced under normal field conditions. A layout of five treatments, with six replicates of each, was set out on land at Avondale, Salisbury, and some of the treatments were repeated at an experimental site on a nearby farm. All beds were burnt and fertilized, but in this case, on the beds at Avondale, a different mixture was used, rated at 12 (P_2O_5), 3 (N_2), and 8 (K_2O).

The beds were sown at the same rate as in earlier trials, the actual amount being 0·63 g. per plot of 24 sq. ft. Virginia tobacco seed, variety 'White Stem Orinoco' was used, which had been cleaned, and treated against seed-borne diseases. The seed was mixed with soil and hand-broadcast, then watered in, on the day following the insecticidal treatments.

Table 2. *Treatments of Avondale series of beds*

	Dosage (lb./acre)		
Bed series	As 2% B.H.C. dust	As B.H.C. technical	As γ-isomer
A, 1–6	0	0	0
B, 1–6	80	1·6	0·19
C, 1–6	160	3·2	0·385
D, 1–6	320	6·4	0·77
E, 1–6	640	12·8	1·54

The B.H.C. was applied as a 50% wettable powder containing 6% γ-isomer, i.e. 12% γ-content in the technical B.H.C. The application rates are detailed in Table 2. All sprays were applied at the rate of 250 c.c. per plot of 24 sq.ft., equivalent to 100 gal./acre. The two higher doses were repeated as part of a further experiment laid out at a farm, but under slightly different conditions. The farm soil was covered over with an inch-thick layer of 'ant-heap' (termitarium), which is of a heavy consistency, and more moisture-retaining than the soil of which the seed-beds were composed. This is a very common commercial practice in Rhodesia. The seed-beds at the farm site were sown to Virginia tobacco, variety Jamaica Wrapper, also treated against seed-borne diseases.

The doses applied to plot series *D*, *E* and *F* are in excess of those which would normally be applied to the seed-beds for control of soil insect pests, but the trend in Southern Rhodesia is to develop permanent sites for the tobacco seedlings, and since the persistence of B.H.C. in the soil under Rhodesian conditions has not been determined, it was thought advisable to find the maximum dose that was safe, or the minimum that would cause serious damage to the seedlings.

Observations of the beds were made every few days, and the following records obtained:

Avondale series (pre-sprayed 28 September 1948, sown 29 September 1948). The initial germination was uneven within the confines of the individual plots, but there were no great differences in germination either within or between treatments. Twelve days after sowing, growth and germination were approximately equal on all plots, and no difference could be observed between those receiving the highest dosage and the controls.

Fifteen days after sowing, a detailed examination was made of batches of seedlings from all plots, to see if any of the seedlings showed the characteristic morphological changes associated with seedlings germinating in contact with B.H.C. Treatment *E* showed seedlings with these typical symptoms, in large numbers. Treatment *D* also had a considerable number of abnormal seedlings with reduced root development. On treatments *B* and *C* there were occasional seedlings showing the symptoms, but these are believed to have occurred only where irregular application (by low-pressure spray as formerly) and/or redistribution by watering had produced spots of locally high concentrations of B.H.C. On treatment *B* the vast majority of the seedlings were normal in appearance.

Twenty-three days after sowing, the *E* plots were noted to be conspicuously poor in growth and plant stand, with no seedlings developed beyond the stage of two true leaves. In complete contrast, the *A* and *B* plots had strongly growing four-leaf stage seedlings, and the *C* and *D* series occupied an intermediate position in regard to plant growth. Forty days after sowing the largest plants were those on the *A* (control) series. Poor growing conditions and waterlogging of the beds with heavy rain made later observations unreliable.

On 8 November 1948, 40 days after sowing, a record was made of the plant stand,

and plant growth on all plots, according to the following scale: 1, nil; 2, very poor; 3, poor; 4, fair; 5, fairly good; 6, good; 7, very good; 8, excellent. These results (Fig. 5) show a definite positive correlation between the dosage of B.H.C. applied before sowing, and the poorness of the growth and stand 40 days later.

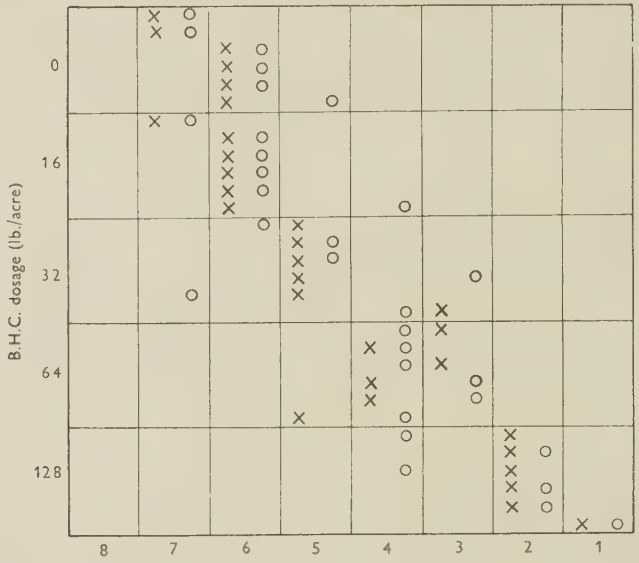

Fig. 5. Effect of B.H.C. on growth (×) and stand (○) of seedlings. Explanation in text. See also Table 4.

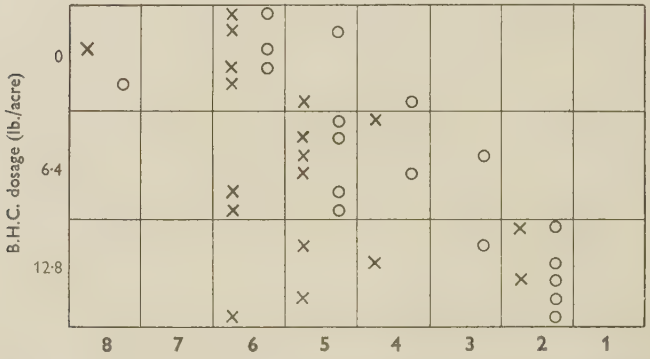

Fig. 6. Effect of B.H.C. on growth (×) and stand (○) of seedlings. Explanation in text.

Farm series. These were pre-sprayed at the two higher doses of B.H.C., with controls, and six replicates of each. The results were substantially in agreement with those obtained from the Avondale series of beds. At this site, however, the beds did

not get waterlogged, and observations were extended over a longer period (Fig. 6). The same correlation is apparent, allowing for the better growing conditions on the farm. The diagrams in Fig. 7 show seedlings taken from the highest dosed B.H.C. series at the farm site. They show the typical stunting of the radicle, and may be compared with Fig. 2, the laboratory-germinated seedlings.

Although during the first few weeks after sowing, the stunted seedlings lagged behind the controls to a considerable extent, later on at the farm site the reduction of competition between the seedlings for space and light, and perhaps for water and fertilizer, began to be apparent. This was especially noticeable on the plots dosed at 12·8 lb. of B.H.C. technical per acre, where most of the seedlings had been eventually killed. The remaining plants on these plots were bigger, stronger, darker in colour, and generally more vigorous and healthy than the control plants, and plants on adjoining commercial seed-beds. A further indication of vigour was given by the almost complete absence of Frogeye lesions (*Cercospora nicotianae*) on these plants, whereas plants on neighbouring plots were heavily or moderately infected.

Fig. 7. Transverse lines denote soil level. Further explanation in text.

The inference from these experiments is that B.H.C. applied at dosages above about 3 lb./acre of the technical product and in the manner described is definitely harmful to the growth of tobacco seedlings. It appears also that lower dosages may produce local phytotoxic effects in parts of the seed-bed.

Trials with parathion and toxaphene as pre-sowing sprays

Alongside the B.H.C. experiment at the farm, detailed above, were carried out trials with three doses of parathion and five doses of toxaphene, applied before sowing the tobacco seed. The toxaphene was sprayed on as a suspension of a 25% wettable powder, and the parathion was hand-broadcast as a 2% dust. Six replicates were treated at each dosage, and the plots were all randomized in blocks. Each plot was 1/1818 acre, or 24 sq.ft., and the application was as before at 100 gallons/acre (Table 3).

The parathion was applied on 30 September 1948, and the toxaphene on 7 October 1948. The beds were sown to Virginia tobacco (Jamaica Wrapper variety) on the day after treatment, in each case. Observations were made over a period of 78 days on all beds, and germination and subsequent growth were quite normal on all the beds dosed with toxaphene. The beds were graded for stand of plants and growth after 41 days, and the results shown in Fig. 8, as for the Avondale and other B.H.C. series of seed-beds.

TABLE 3. *Schedule of parathion and toxaphene seed-bed treatments*

Parathion series:	Dosage (lb./acre)	
Beds	As 2 % dust	As active parathion
A	0	0
D	24·0	0·48
E	48·0	0·96
F	96·2	1·8

Toxaphene series:	Dosage (lb./acre)	
Beds	As 25 % wettable powder	As toxaphene
A	0	0
B	1·60	0·40
C	3·20	0·80
D	6·40	1·60
E	12·80	3·20
F	25·60	6·40

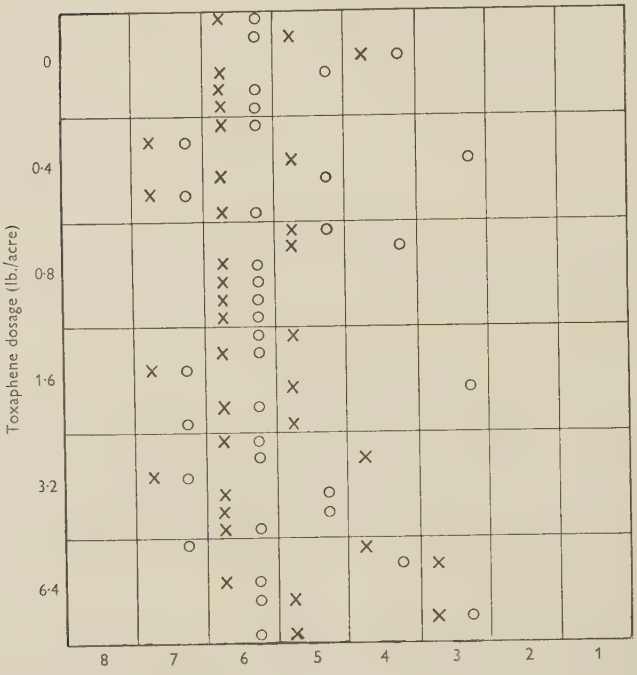

Fig. 8. Effect of toxaphene on growth (×) and stand (○) of seedlings. Explanation in text. See also Table 3.

In the case of the parathion-treated plots, the results were the same. Fig. 9 shows a correlation between dose and growth and stand of the seedlings 48 days after

sowing, and no depressant action appears to have occurred. The maximum dose was far less than that applied in the experiment described above where parathion caused marked stunting of the seedlings: the dose there was over 22 lb./acre of active parathion, as compared with 1·8 lb. applied to the other seed-bed plots.

It is inferred, therefore, that at normal doses, no harm is to be expected from the application of parathion or toxaphene as soil insecticides on tobacco seed-beds.

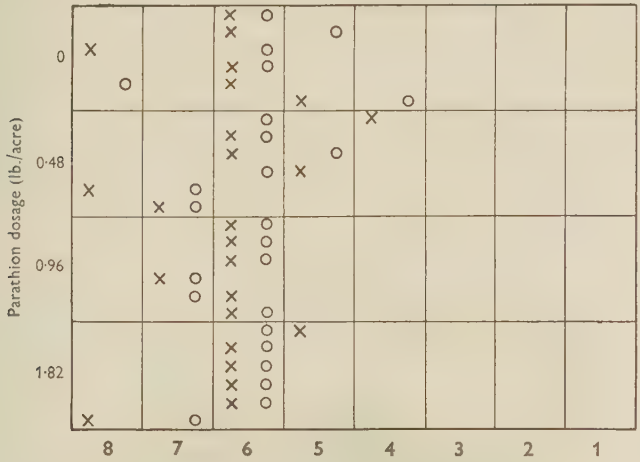

Fig. 9. Effect of parathion on growth (×) and stand (○) of seedlings. Explanation in text. See also Table 3.

Experiments on re-sowing the treated beds at the farm site

About mid-December 1948, all the experimental beds were cleared, and on 11 January 1949 they were re-sown; in addition, a half-quantity of fertilizer was applied. Nine days after sowing, germination was even and satisfactory on all plots, including those treated with B.H.C. at 6·4 and 12·8 lb. technical B.H.C. per acre. Sixteen days after sowing, there were still no differences between treatments, and no seedlings with stunted roots on the B.H.C. plots. Thirty-three days after sowing all seedlings were normal in size and were vigorous and healthy.

Since B.H.C. has been reported to persist in soil (Smith, 1948*a*) for many months under certain conditions these results were unexpected. However, the soil was disturbed during the clearing of the beds, and it is probable that the second lot of seeds germinated quite out of contact with the B.H.C.-containing soil. The seeds are not raked in after sowing, but left on the soil surface to germinate, and unfortunately these results cannot be taken to show that the B.H.C. had lost its activity: there is still the possibility that repeated applications year after year may build up a toxic deposit in the soil.

Mortality of insects on the Avondale series of B.H.C.-treated beds

Complementary to the phytotoxicity tests at Avondale, records were also kept of the insecticidal activity of the B.H.C. The plots were inspected three times for dead insects, and the counts made for the various treatments, compared with the different doses of B.H.C., are shown in Fig. 10. The lower graphs (I, II and III) show the

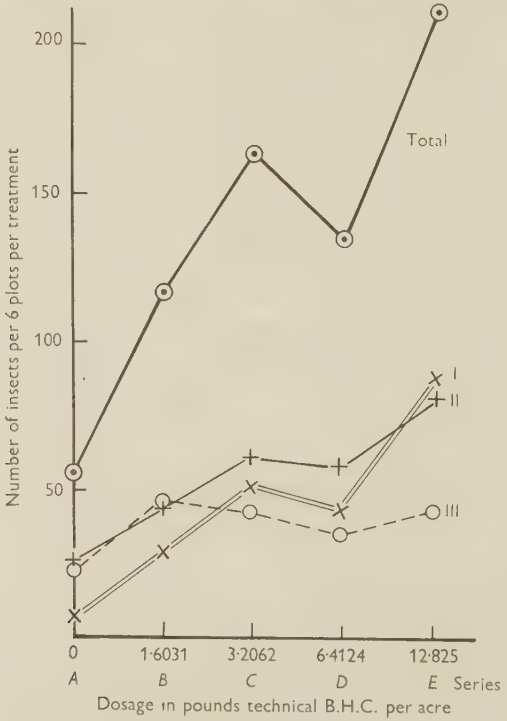

Fig. 10. Explanation in text.

actual numbers of dead insects collected on each occasion, 16, 29 and 42 days after treatment. It is significant that the insecticidal powers of the B.H.C. persisted for 6 weeks, as considerable numbers of the insects taken at the last count were freshly killed. No records could be taken after this date, as the beds were washed over by heavy rains.

The phytotoxicity of B.H.C. to the later stages of tobacco

In October 1948, reports were received from a number of growers of a new 'disease' of tobacco in the late seedling stage, causing a distortion of the leaves, a slowing-down of growth, and in extreme cases death of the young plants. Typical symptoms

were as follows: a thickening of the third and/or fourth true leaves, with the first and second true leaves normal in appearance; the veins on the affected leaves were distorted from their usual pattern, and a notching or sinuation of the lamina occurred ('oak-leaf' symptom). Slightly affected plants grew away from the distortions without apparent ill-effects, and no further symptoms developed in the newly formed leaves. On transplanting into the field, subsequent growth of these plants was completely normal.

The symptoms were in all cases associated with the application to the seedlings of varying amounts of dusts of from 3 to 5% technical B.H.C. content. This treatment had been found satisfactory for the control of various seed-bed pests, in particular lepidopterous larvae or 'cutworms'. It was not, however, clear why the symptoms were not universal, since only a small proportion of the seed-beds receiving this treatment were affected. Experimental proof of the ability of B.H.C. to produce the typical symptoms was therefore sought.

A normal commercial seed-bed, of even stand and growth, was used for the application of graded doses of B.H.C. Plots, each 2 sq.yd. in extent (no replicates) were used, and each treated plot was separated from the next by a control plot of the same size. The pH of the bed was 8·4, and this had been ascertained previously to be normal for heavily burnt seed-beds (surface soil).

The tobacco in the bed was Virginia (Bonanza variety), and was sown on 11 October 1948. The B.H.C. was applied on 1 November 1948, the tobacco being then 11 days after germination.

The B.H.C. was applied as a 3% dust or a 50% wettable powder, in each case the γ-isomer content being 10% of the crude B.H.C. The dosages are given in Table 4.

TABLE 4. *Schedule of doses of B.H.C. applied to 11-day-old tobacco*

Plot	Treatment	Dosage (lb./acre)	
		As 3% dust	As technical B.H.C.
A	¼ oz. 3% B.H.C. dust per sq.yd.	75	2·25
B	½ oz. 3% B.H.C. dust per sq.yd.	150	4·50
C	1 oz. 3% B.H.C. dust per sq.yd.	300	9·00
D	¼ oz. 50% B.H.C. wettable powder per sq.yd.	1250	37·5

All dusts included Fuller's earth as filler and the plots were evenly dusted by hand, and watered immediately afterwards.

Eleven days after treatment, at all dosages, the plants on the treated plots were smaller than those on the controls, but no sinuation of the leaves was visible. Seventeen days after treatment, the presence of 'oak-leaf' symptoms on all treated plots was noticed, but none on any control plots. A heavy mortality of plants occurred on the plot treated with 37·5 lb. of B.H.C. (technical) per acre, but a considerable number survived. Twenty-five days after treatment all surviving plants

were growing vigorously. The plots with the three lowest doses of B.H.C. still lagged behind the control plots, and sinuate-edged leaves were still visible.

A second trial was made on older seedling tobacco, the B.H.C. being applied at the same rates, and the plants of age 21 days after germination. Application was exactly as for the first experiment, but (observations being taken 6 and 14 days after treatment) the effect of the B.H.C. was reduced to some stunting and slowing of growth at the two highest doses only. Apparently, the older the plant, the greater its resistance to the effects of B.H.C.

Fig. 11. A, B.H.C.-distorted plant; B, C, typical 'oak-leaf' distortion; D, younger leaf produced above distorted leaves. (All traced, × ¼.)

Discussion of the effects of B.H.C. on tobacco seedlings

Whilst it is now shown that tobacco seedlings treated with B.H.C. in the manner described exhibit characteristic symptoms above a certain dosage, and vary in their susceptibility to B.H.C. with age, it is not known what is the mechanism of this toxic action. Although heavy doses of B.H.C. will kill many young seedlings, others survive, a fact which is not readily explained. The characteristic symptoms appear on 11-day-old seedlings at 2·25 lb. technical B.H.C. per acre, and are successively more marked with higher doses. The distorted leaves are quite healthy in appearance, the same colour as normal leaves.

These symptoms may be evoked by B.H.C., as by other factors, or they may be a specific reaction to B.H.C. The action is connected with the development of the plant, as if the B.H.C. blocked some process temporarily, and was gradually eliminated as the plants grow older. This is shown by new leaves formed on symptomatic plants failing to show distortion. The increasing resistance with age may perhaps be due to a lesser degree of entry into the plant. The mode of entry is not known: tobacco plants (about 7 weeks old) transplanted into holes containing 10 g. of 3% B.H.C. dust have grown without showing any symptoms. Preliminary trials with the injection of B.H.C. suspensions just below the bud in transplanted tobacco have failed to evoke symptoms in the new growth. Potted seedling plants

dusted with B.H.C. at rates which produce distortion in seed-beds have not shown symptoms: this may be connected with the check in growth at transplanting. There appears to be a definite connexion between the growth rate of the plant and its susceptibility to B.H.C.

Fig. 11 shows typical seedling distortion, and the resemblance of the affected leaves to those of an oak.

The writer gratefully acknowledges the assistance and co-operation of the tobacco farmers who generously allowed experiments to be performed on their land: acknowledgements are also extended to the President and Secretary of the Rhodesia Tobacco Association for permission to publish this paper; to members of the Southern Rhodesian Government Scientific Departments for their co-operation, and to Dr E. Parry Jones, Director of the Research Scheme, for helpful advice and criticism. In addition, technical assistance from Messrs J. S. Riley, E. T. M. Reid, and P. W. Miles has been of the greatest value.

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(*Received* 29 *March* 1950)

ROGUING POTATO CROPS FOR VIRUS DISEASES

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(With 2 Text-figures)

Removing virus-infected plants from plots of Majestic potatoes at Rothamsted on 2 July 1947 did not reduce the spread of leaf roll but reduced rugose mosaic (potato virus *Y*) to about one-fifth of that in plots rogued on 21 July or left unrogued. Roguing Arran Pilot potatoes on 16 June or 2 July reduced leaf roll to five-sixths of that in unrogued plots; roguing on 16 June reduced rugose mosaic to about half that in plots rogued on 2 July, and about a quarter of that in unrogued plots. Lifting Arran Pilot potatoes in mid-August reduced virus diseases to about two-thirds.

Roguing flattened the gradient (decrease in percentage plants diseased with increasing distance from the source of infection) with rugose mosaic, but had little effect with leaf roll. Evidently any plants prevented by roguing from contracting virus *Y* were near the initially infected plants.

In 1948, Majestic and King Edward potatoes at three places were rogued during 22–24 June and tubers were dug during 28–30 July and again at the end of the season. Leaf roll spread more in Majestic than in King Edward, and rugose mosaic spread more in King Edward. Roguing reduced the spread of both by about one-fifth at Rothamsted, but had no effect at Sutton Bonington. At Bretton, in the Derbyshire hills, roguing had no effect on leaf roll, but prevented the spread of rugose mosaic.

The small benefit occasionally achieved by roguing in the ware-growing districts of England does not make the practice economically worth while.

Experiments on the effect of roguing plants infected with potato virus *Y* (causing rugose mosaic) and leaf roll virus from potato crops have been made at Rothamsted since 1943 (Doncaster & Gregory, 1948; Gregory, 1948*a*). The present paper describes one experiment made at Rothamsted in 1947 and another, with which the series finished, made at three places in 1948. The 1947 experiment tested the effects of early and late roguing on the health of tubers dug at the end of September from a crop of the variety Majestic, and of tubers dug in mid-August and the end of September from a crop of the variety Arran Pilot. The 1948 experiment tested the effect of roguing on the health of tubers of the varieties Majestic and King Edward, dug at the end of July and at the end of the season.

The 1947 experiment

The experiment consisted of forty plots, of which four were controls, having no added infected plants. The other plots were in six randomized blocks of six plots each, four blocks planted with stock seed Majestic and two with stock seed Arran

Pilot potatoes. Each plot was approximately square (sixteen rows of twenty-five plants) with eight virus-infected plants of the variety King Edward in positions 7 and 19 in rows 3, 7, 11 and 15 (Fig. 1). The plots were planted on 8–9 May 1947 in a crop of 'A' stock Majestic potatoes.

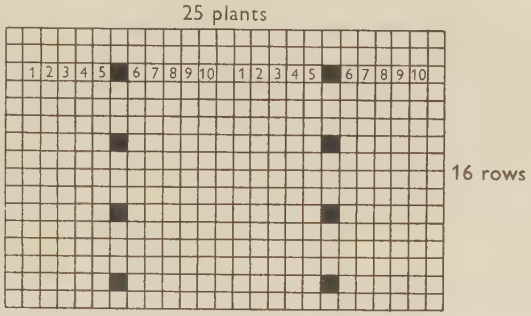

Fig. 1. Roguing experiment, Rothamsted, 1947. Plan of a single plot, showing positions of infected King Edward plants (shaded). 1–5, 6–10 = plants sampled on each side of infected plants.

The treatments applied were:

	Arran Pilot	Majestic
Roguing dates (two plots of each per block)		
Early	16 June	2 July
Late	2 July	21 July
Not rogued		
Sampling dates		
	15–20 Aug.	30 Sept.
	30 Sept.	

In sampling, a single tuber was taken from each of the five plants on either side of the infected plant in the same row. These were replanted the following year, and were examined later for health. The four control plots were sampled on 30 September by taking one tuber from each of 100 plants selected at random.

The potatoes were planted late because of severe weather during the first four months of 1947. Emergence of both Arran Pilot and Majestic was complete by about 16 June. The weather during the summer was very hot and dry.

Aphids infesting the potatoes were counted at intervals and sticky traps were set up in the crop. The data obtained have been given by Broadbent (1948*a*, *b*). *Aphis rhamni* Fonsc. multiplied rapidly during the last fortnight of July, and reached a maximum population at the end of the month. This species was more numerous on Majestic than on Arran Pilot potatoes. *Myzus persicae* (Sulzer) were few until mid-July, when large numbers of alatae entered the crop. The population increased rapidly during the next fortnight, and the summer dispersal migration from the crop

took place during the last few days of July and the first fortnight of August. The population then remained low until near the end of the season, when it again increased slightly before the autumn migration from the crop.

Records of virus spread

The percentages of infected tubers in the various samples (Table 1) were determined by planting in the following year.

Variety Majestic. About 80–90% of all samples were infected with leaf roll virus, and roguing had no effect. Roguing on 2 July, however, reduced the incidence of rugose mosaic to about one-fifth of that in plots rogued on 21 July or left unrogued. There was no difference in rugose mosaic between plots rogued on 21 July and those not rogued, so most of the spread of virus *Y* must have occurred between 2 July and 21 July.

Variety Arran Pilot. About 50–60% of all tubers dug at the end of the season produced plants with leaf roll despite roguing, but in the plots left unrogued there was 87% leaf roll in the plant-position next to the 'infector'. In other positions roguing had no effect on leaf roll. Roguing on 16 June reduced the incidence of rugose mosaic to about half that in plots rogued on 2 July, and to about a quarter of that in plots left unrogued. Early lifting reduced the infection with both viruses in all plots.

The small amount of rugose mosaic in the plots rogued early showed that little spread of this disease resulted from the activity of the few early migrant aphids which visited the crop in June, and that most of the spread must have taken place after 2 July. No leaf drop streak was seen when the plants were examined on 21 July, but symptoms had appeared by 8 August, by which date the rugose mosaic 'infector' plants were beginning to die. Many of the infections must have taken place about mid-July. As roguing did not reduce the spread of leaf roll, this either took place before July from infected plants in the plots (which is doubtful as there were few aphids during this period), or the virus was spread over all the plots by the large numbers of alatae which visited the field in mid-July (or by the progeny of these alatae). It is not known whether the alate *M. persicae* brought leaf roll virus into the field from elsewhere or spread it from infected plants already in the field. The high percentage of plants infected with leaf roll virus, without 'gradient' (decline in percentage infection with increasing distance from the source of infection), suggests that many plants contracted the virus from sources outside the crop.

Significantly fewer of the Arran Pilot potatoes lifted in August were infected with leaf roll virus than those lifted at the end of September. The numerous plants infected in July might have acted as sources of infection by September, but whether the late spread was the result of activity by apterae or by alatae is not known, though the increase in population at the end of September following a renewal of the trap catches of *M. persicae* between 8 and 22 September makes both possible.

TABLE 1. *Percentages of plants infected, Rothamsted* 1947

Majestic potatoes

Plant positions from 'infector'	Rogued 2 July	Rogued 21 July	Not rogued	Control
		Rugose mosaic		
1	4	44	47	
2	5	30	25	
3	4	13	11	
4	3	9	10	
5	3	9	3	
Mean	4	21	19	5
		Leaf roll		
1	95	89	89	
2	86	93	83	
3	90	89	88	
4	89	85	85	
5	84	88	90	
Mean	89	88	87	82

Arran Pilot potatoes

	Rogued 16 June	Rogued 2 July	Not rogued	Control
		Lifted 15–20 August		
		Rugose mosaic		
1	5	22	54	
2	5	8	24	
3	5	10	9	
4	2	2	9	
5	2	5	10	
Mean	4	9	22	—
		Leaf roll		
1	37	47	61	
2	40	39	53	
3	33	34	56	
4	22	35	42	
5	37	27	31	
Mean	34	36	48	—
		Lifted 30 September		
		Rugose mosaic		
1	13	31	61	
2	16	26	32	
3	5	17	22	
4	5	14	22	
5	12	5	20	
Mean	10	18	31	8
		Leaf roll		
1	55	67	87	
2	41	54	66	
3	60	62	60	
4	54	49	57	
5	48	53	63	
Mean	52	57	66	55

Spatial analysis of 1947 data

Earlier experiments in this series were too small to show any effect of roguing on infection gradients, but the 1947 experiment was large enough for the effects to be measured, using the method described by Gregory & Read (1949). The percentage of diseased plants was first subjected to the multiple infection transformation (Gregory, 1948*b*) to obtain I, the 'expected' number of infective aphid punctures per 100 plants. The values of I in different plant positions were used to calculate $\log_{10} \hat{I}_1$, the estimate for $\log_{10} I$ at 1 m. from the source, and b (m^{-1}), the gradient per metre.

Variety Majestic. The effects of roguing date on rugose mosaic, in terms of the number of infective punctures estimated at 1 m. from the source, $\log_{10} \hat{I}_1$, and the decrease in this number per metre at increasing distances, b, are shown in Table 2.

Table 2. *Effect of roguing date on rugose mosaic in Majestic potatoes*

	$\log_{10} \hat{I}_1$			b (m^{-1})		
	Plant positions			Plant positions		
Treatment	1–5	6–10	Mean	1–5	6–10	Mean
Rogued 2 July	0·63	0·57	0·600	−0·06	−0·17	−0·115
Rogued 21 July	1·36	1·41	1·385	−0·60	−0·46	−0·530
Not rogued	1·30	1·35	1·325	−0·82	−0·71	−0·765
Mean difference:						
2 July v. later*			0·755 ± 0·032		0·53 ± 0·063	
21 July v. not rogued			0·06 ± 0·037		0·235 ± 0·073	

* 'later' = mean of '21 July' and 'not rogued'.

The effects of early roguing on intensity at 1 m., and on the gradient, were significant, but late roguing had no effect on intensity and only a small effect on gradient.

Roguing had no effect on either the intensity or gradient of leaf roll. There were on the average about 200 infective punctures per 100 plants, with little or no gradient (Fig. 2).

Variety Arran Pilot. The effects of roguing date on rugose mosaic and leaf roll are summarized in Table 3.

Early roguing had a significant effect on the incidence of rugose mosaic and of leaf roll at 1 m., but not on the gradient. Plots rogued on 16 June showed only 33% of the rugose mosaic and 72% of the leaf roll found in those rogued on 2 July or left unrogued. Other comparisons were not statistically significant. The effect of early lifting of Arran Pilot was shown (Table 4) to reduce the incidence of both rugose mosaic and leaf roll slightly; but except for the intensity of leaf roll the effects were not statistically significant.

Two distinct results of early roguing can thus be recognized. Roguing early enough reduces the number of infective aphid punctures at the standard distance of

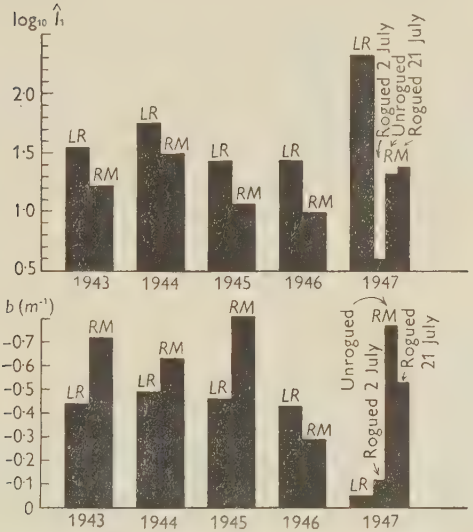

Fig. 2. Log of estimated infective punctures per 100 plants at 1 m. from source of infection ($\log_{10} \hat{I}_1$) and change of $\log_{10} \hat{I}$ per metre [b (m^{-1})]. Data from Majestic potato-roguing trials at Rothamsted, 1943–7.

TABLE 3. *Effect of roguing date on rugose mosaic and leaf roll in Arran Pilot potatoes*

(S.E. based on 3 D.F.)

	$\log_{10} \hat{I}_1$			b (m^{-1})		
	Plant positions			Plant positions		
Treatment	1–5	6–10	Mean	1–5	6–10	Mean
			Rugose mosaic			
Rogued 16 June	0·85	0·87	0·86	−0·23	−0·21	−0·22
Rogued 2 July	1·17	1·22	1·195	−0·66	−0·31	−0·485
Not rogued	1·37	1·59	1·48	−0·33	−0·52	−0·425
Mean difference:						
16 June v. later		0·48 ± 0·089			0·235 ± 0·13	
2 July v. later		0·29 ± 0·102			−0·06 ± 0·15	
			Leaf roll			
Rogued 16 June	1·71	1·80	1·755	0·00	−0·05	−0·025
Rogued 2 July	1·79	1·85	1·82	−0·08	−0·17	−0·125
Not rogued	1·99	1·95	1·97	−0·24	−0·16	−0·20
Mean difference:						
16 June v. later		0·14 ± 0·04			0·14 ± 0·05	
2 July v. later		0·15 ± 0·05			0·07 ± 0·05	

TABLE 4. *Effect of lifting date on rugose mosaic and leaf roll in Arran Pilot potatoes*

	$\log_{10}\hat{I}_1$			$b\ (m^{-1})$		
	Plant positions			Plant positions		
Treatment	1–5	6–10	Mean	1–5	6–10	Mean
			Rugose mosaic			
Lifted 15–20 Aug.	0·94	1·18	1·06	−0·40	−0·52	−0·46
Lifted 30 Sept.	1·35	1·40	1·375	−0·37	−0·28	−0·325
			Leaf roll			
Lifted 15–20 Aug.	1·72	1·74	1·73	−0·15	−0·17	−0·16
Lifted 30 Sept.	1·93	1·99	1·96	−0·08	−0·10	−0·09

1 m., and flattens the rugose mosaic gradient. The difference between rogued and unrogued plots indicates that early infections occur nearer the source of infection than later ones. The effect of early lifting apparently differed from that of roguing, as it reduced the spread of both diseases without flattening the gradient. On the other hand, there is some evidence that early lifting makes the gradient of both diseases steeper.

Gradients are calculated on distances measured in metres in order to facilitate comparisons between different pathogens. It may be that gradients of insect-borne viruses depend on plant spaces and not on absolute distances, but this will be most readily recognized when gradients for different spacings are compared on a distance basis.

ROGUING EXPERIMENTS IN 1948

Experiments on a uniform plan were made at three places: (1) Rothamsted, Hertfordshire, (2) Sutton Bonington, Nottinghamshire, (3) Bretton, Derbyshire, the last being on an exposed ridge at an altitude of 1150 ft.

At each place sixteen plots were arranged in a Latin square, with two guard rows between plots in one direction and three guard plants in the other. The plots were surrounded by healthy potatoes. Each plot consisted of five rows of fourteen plants. Half the plots were of the variety King Edward (stock seed), half of Majestic (stock seed). The fourth plant from each end in every row was virus infected (Table 5). The infected plants were removed from eight of the plots in June, the other eight

TABLE 5. *Plan of a single plot*, 1948

(0, 1, 2, 3 = healthy plants; 1, 2, 3 sampled. *L* = leaf roll, *Y* = rugose mosaic.)

3	2	1	*L*	1	2	3	0	3	2	1	*Y*	1	2	3
3	2	1	*L*	1	2	3	0	3	2	1	*Y*	1	2	3
3	2	1	*L*	1	2	3	0	3	2	1	*Y*	1	2	3
3	2	1	*L*	1	2	3	0	3	2	1	*Y*	1	2	3
3	2	1	*L*	1	2	3	0	3	2	1	*Y*	1	2	3

being left unrogued. At the time of roguing, leaf roll and rugose mosaic were visible in the infected plants at Rothamsted and Sutton Bonington, but not at Bretton. At the end of July and again at the end of the season, the plots were sampled by taking one tuber from each of three initially healthy plants on each side of the infected plant in the same row. The size of haulms differed greatly at the different centres; at the end of July the Rothamsted plants were intermediate between those at Sutton Bonington, which were long and intertwined, and those at Bretton, which were short so that plants in adjacent rows scarcely touched. The dates of planting, roguing and sampling were:

	Planting	Roguing	Sampling 1st	Sampling 2nd
Rothamsted	16 Apr.	22 June	28 July	29 Sept.
Sutton Bonington	12 Apr.	24 June	29 July	9 Sept.
Bretton	19 Apr.	23 June	30 July	14 Oct.

At Rothamsted every plant was infested with *Myzus persicae*, *Macrosiphum euphorbiae* (Thomas) and *Aphis rhamni* by the middle of July, but by the end of the month, and for the rest of the season, aphids were scarce. Frequent counts could not be made at the other places. At the time of roguing in June, *Myzus persicae* and *Macrosiphum euphorbiae* were numerous at Sutton Bonington, but no *Aphis rhamni* were found. A few *Myzus persicae* were counted at Bretton. At the end of July *M. persicae* and *Macrosiphum euphorbiae* were fairly numerous at both places.

TABLE 6. *Sticky trap catches of* Myzus persicae, 1948

	Before roguing (24 June)	24 June to 1st sampling (30 July)	30 July to 2nd sampling	Total
Rothamsted	2	20	3	25
Sutton Bonington	18	28	21	67
Bretton	0	2	12	14

Sticky traps were erected in each experiment (Table 6). The aphid infestation at Bretton was much later than at the other places, and trap catches of potato aphids also occurred late in the season.

Records of virus spread, 1948

The percentages of infected tubers are given as means for rogued and unrogued plots in Tables 7 and 8. On the average the percentage of leaf roll was higher in Majestic than in King Edward, whereas rugose mosaic was higher in King Edward than in Majestic. There was little difference at the end of July between rogued and unrogued plots, which suggests that most of the spread of virus had occurred before the plots were rogued in the fourth week of June. But in each of the three places there was an increase in infection between the first and second samplings. Late infections, in the rogued plots, probably came partly from plants infected early in the

season and partly from the infected plants in the unrogued plots. The virtual absence of potato aphids after the end of July at Rothamsted suggests that most of the season's spread had taken place before the plots were sampled on 28 July, but that not all infections had become systemic.

Table 7. *Average percentage infection in three plants on either side of the diseased plant in rogued and unrogued plots*, 1948

	Sampling date	Rogued			Not rogued		
		Majestic	King	Edward	Majestic	King	Edward
		Rugose mosaic					
Rothamsted	28 July	16		15	23		13
	29 Sept.	30		35	41		40
Sutton Bonington	29 July	23		19	27		30
	9 Sept.	37		43	36		47
Bretton	30 July	2		0	0		1
	14 Oct.	1		0	6		21
		Leaf roll					
Rothamsted	28 July	43		35	49		35
	29 Sept.	54		49	60		60
Sutton Bonington	29 July	84		62	75		81
	9 Sept.	94		86	89		84
Bretton	30 July	17		13	19		24
	14 Oct.	50		31	44		33

Table 8. *Percentages of infected tubers*, 1948. (*Majestic and King Edward results pooled*)

	Sampling date	Rogued plots				Unrogued plots			
		Plants from infector				Plants from infector			
		1	2	3	Mean	1	2	3	Mean
		Rugose mosaic							
Rothamsted	28 July	21	14	11	15	19	16	19	18
	29 Sept.	41	34	21	32	53	34	34	40
Sutton Bonington	29 July	40	13	12	21	42	23	23	29
	9 Sept.	52	31	36	40	47	38	36	40
Bretton	30 July	1	2	0	1	1	1	1	1
	14 Oct.	1	0	0	1	23	12	6	13
		Leaf roll							
Rothamsted	28 July	50	39	28	39	50	42	35	42
	29 Sept.	60	50	44	51	64	58	59	60
Sutton Bonington	29 July	83	71	67	73	82	77	75	78
	9 Sept.	92	93	86	90	87	87	88	87
Bretton	30 July	18	18	9	15	24	15	26	21
	14 Oct.	46	36	40	40	49	33	33	38

The only substantial difference between plots rogued and plots left unrogued was at Bretton, where the increase of rugose mosaic which occurred after roguing was confined to the unrogued plots.

In general, a larger proportion of the season's spread of leaf roll than of rugose mosaic had occurred by the time of the first sampling (Table 9). Late spread of rugose mosaic, compared with leaf roll, has been unusual in most years in the ware-growing areas.

TABLE 9. *The percentages of the season's spread of rugose mosaic and leaf roll before 28–30 July 1948*

	Rothamsted	Sutton Bonington	Bretton
Rogued plots			
Rugose mosaic	47	53	100
Leaf roll	77	81	38
Unrogued plots			
Rugose mosaic	45	73	8
Leaf roll	70	90	55

TABLE 10. *Relative number of infective aphid punctures and of* Myzus persicae *trapped*, 1948

	Rugose mosaic	Leaf roll	*M. persicae*
Bretton	1·0	1·0	1·0
Rothamsted	3·6	1·9	1·8
Sutton Bonington	3·6	4·3	4·8

The percentages of infected plants in the unrogued plots were transformed to estimated number of infective aphid punctures per 100 plants (Gregory, 1948*b*), and these estimates are given as ratios in Table 10, the figures for Bretton being regarded as unity. Leaf-roll infection, but not rugose mosaic, was correlated with the number of *M. persicae* trapped during the season, as in previous years (Broadbent, 1950). Bawden & Kassanis (1947) found nine species of aphids capable of transmitting virus *Y*, the most efficient in the glasshouse being the three common potato aphids. However, the vectors of non-persistent viruses need not be insects which normally colonize the plants (Dickson, Swift, Anderson & Middleton, 1949), and during the season aphids of many species visit the plants (Broadbent, 1948*a*). It is possible, therefore, that potato virus *Y*, which is non-persistent in its vectors, is spread partly by aphids which do not breed on potato. The gradients suggest that most flights by aphids within the field are short, from plant to plant.

CONCLUSION

The results of roguing experiments with Majestic potatoes at Rothamsted can be briefly summed up as follows:

1943. Roguing on 4 July had no effect.

1944. Roguing on 19 June, before all disease symptoms were showing, caused a

substantial reduction in incidence of rugose mosaic, but no reduction of leaf roll. Roguing on 19 July had no effect.

1945. Roguing on 16 July slightly reduced the incidence of leaf roll only.

1946. Roguing on 14 June, before symptoms were showing, reduced the final incidence of leaf roll by about half, but had no effect on rugose mosaic. Roguing on 6 July and on 27 July had no effect.

1947. Roguing on 2 July substantially reduced the incidence of rugose mosaic only. Roguing on 21 July had no effect.

1948. Roguing on 22 June slightly reduced the incidence of both diseases.

It is obvious that in most years a large proportion of the season's spread of viruses occurs before disease symptoms are recognizable, and thus before roguing can take place. Under these circumstances, roguing in the south and east of England is not worth while.

We are indebted to Mr D. R. Read, of the Statistics Department, Rothamsted Experimental Station, for the analysis of the 1947 results.

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(*Received 23 March* 1950)

THE DISTRIBUTION OF APHID INFESTATION IN RELATION TO LEAF AGE

I. *MYZUS PERSICAE* (SULZ.) AND *APHIS FABAE* SCOP. ON SPINDLE TREES AND SUGAR-BEET PLANTS

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(With 15 Text-figures)

The leaf-by-leaf distributions of free infestations of viviparous *Myzus persicae* (Sulz.) and *Aphis fabae* Scop. were recorded on sugar-beet plants and spindle bushes in pots in the greenhouse, and the distributions of the various seasonal forms of *A. fabae* were recorded on the same plants growing naturally outdoors. The aphid distributions were related to the ages of the leaves estimated on an arbitrary scale of ten types of leaf distinguished by degree of unfurling and colour. The diverse types of distribution recorded were all reducible to a common general pattern: growing and senescing leaves were more susceptible to colonization than maturing, mature and dying leaves. The resultant two-peaked curve of aphid density on a series of leaves at successive stages of development was found in its entirety on certain sugar-beet plants, but part only of the complete curve was usually found, owing to the incomplete range of leaf ages present on any one plant, particularly on the spindle tree.

Fundatrigeniae were found colonizing sugar beet, and spring and summer migrants were found starting colonies on the spindle tree. The normal association of each seasonal form of the aphid either with the 'primary' winter host, or with a 'secondary' summer host, but not with both, is therefore not obligatory. It is attributed to the normal inaccessibility of summer hosts to fundatrigeniae and the normal unsuitability of the winter host when the 'alienicolae' are migrating and its leaves are mature.

When *M. persicae* and *A. fabae* were infesting the same plant at the same time, their distributions were broadly alike, but *M. persicae* extended on to both younger and older leaves than did *A. fabae* and more strictly avoided the more mature leaves. Hence it is suggested that the degree of adaptation of a given aphid to a given plant may be gauged by the extent to which the aphid can colonize the plant's leaves not only when they are growing and senescing but also when they are mature and fully functional.

Introduction

A previous paper (Kennedy & Booth, 1950), describing experimental methods for studying the feeding preferences and relative fecundity of *Aphis fabae* Scop. among leaves of different ages and kinds, gave the theoretical grounds for comparing individual leaves as host-units for the aphids, instead of, or rather as the basis for, comparing whole plants. The observational grounds for doing so are now given in

this and the following paper (Ibbotson & Kennedy, 1950). The experimental work itself, having been planned to confirm and extend the conclusions drawn from these observations of free aphids on whole plants, will be described subsequently.

Material

Two sets of observations were made, indoor and outdoor. In both sets the infestations recorded had developed spontaneously, from free apterae or alatae. The identity of the aphids colonizing the pot plants was not in doubt, for the *A. fabae* came from the mass, clonal culture maintained in the same house (Kennedy & Booth, 1950), and the *Myzus persicae* from a 'rogue' stock which maintained itself on various plants there through the winter–spring 1947–8. On the advice of Dr D. Price-Jones, it was assumed that black aphids found on outdoor sugar beet were only *A. fabae* Scop., and not *A. euonymi* (Börner & Janisch), especially since conspicuous white wax markings were common on the adult apterae. But there was every possibility that the black aphids found on outdoor spindle bushes might include *A. euonymi* as well as *A. fabae*, so that occasional samples of these aphids were collected. Table 1 summarizes the composition of each of the three main types of infestation on the spindle bushes: spring colonies directly descended from fundatrices hatched from eggs; spring and summer colonies mothered by alate 'alienicolae'; and autumn colonies mothered by gynoparae.

Table 1. *Composition of adult black aphid populations found colonizing outdoor spindle trees*, 1948–9

		No. of specimens identified as		
Period	Forms	*A. fabae* Scop.	*A. euonymi* (B. & J.)	Doubtful
April–May	Apterae viviparae (fundatrigeniae)	115	24	11
	Alatae viviparae (progeny of above)	87	3	11
May–August	Alatae viviparae (immigrants)	438	13	9
	Apterae viviparae (progeny of above)	38	0	0
September–October	Alatae gynoparae	134	0	0
	Oviparae	157	0	0

Adult viviparous black aphids with long hairs on the third segment of the antenna and on the marginal sclerites of the abdomen, and with few or no lateral papillae on abdominal segments II–V (not more than one segment with a pair of papillae, plus one or two unpaired ones), were classed as *A. fabae* Scop. Aphids with shorter hairs and six or more papillae on abdominal segments II–V (at least two segments with a pair of papillae) were classed as *A. euonymi* (Börner & Janisch). Intermediate types were classed as 'doubtful'. The spring and summer colonies contained only a small

minority of *A. euonymi* so judged, so that the counts of these populations can be safely said to refer, in the main, to *A. fabae*. The autumn samples contained only *A. fabae*. That the above criteria were reliable as a means of excluding viviparous *A. euonymi* from intended counts of *A. fabae*, appeared likely from the finding that all the oviparae had the paddle-shaped hind tibiae densely studded with sensoria, by which *A. fabae* may be distinguished most certainly from *A. euonymi*.

The sugar-beet plants (*Beta vulgaris* L.) used were of mixed strains of var. Bush 'E', kindly supplied as seed or stecklings by British Pedigree Sugar Beet Seed Ltd. The spindle trees (*Euonymus europaeus* L.) were either established in the garden of the Entomological Field Station, Cambridge, or were transplanted there from a coppice at White Hill, 3 miles south of Cambridge. All but one set (see Table 3) of the observations on outdoor plants were made in the Field Station garden.

Assessment of leaf age

In order to assess the relative age of any given leaf, a standard system was devised, based on certain visual characters common to the leaves of all the plants but different between leaves of successive physiological ages. This typing system was developed by stages in the course of the early observations and experiments, and the final choice of types to form the standard system was decided in the light of the findings, not only concerning plant growth, but also concerning aphid behaviour.

The amounts of growth and senescence in progress could be judged to some extent by the proportions in which the different leaf-types occurred on the plants, and especially by changes in those proportions between two successive typings of the same plant (see pp. 665–7). In addition, the growth rates and rates of colour change of all the individual leaves were recorded from time to time on shoots or whole plants in different general physiological states. Where desired for standardization purposes, the types assigned to the growing leaves could then be corrected according to their actual growth rates, and the types assigned to older leaves could be subdivided according to their rates of senescence (see Ibbotson & Kennedy, 1950; Kennedy & Booth, in press).

Thus all assessment of leaf age was founded upon a primary, arbitrary classification by immediately visible type. The equivalence of the types could later be checked, and their physiological meaning could be further defined to whatever degree the work demanded, by qualificatory information of the kind mentioned. This procedure proved to be the most convenient in practice.

The leaves were classified into three main groups: younger, growing leaves; mature, fully developed leaves; and older, senescent leaves. These three groups were further divided into a total of ten types, each type representing a recognizable stage in the growth or senescence of the leaf.

The growing leaves were divided into three distinct types, according to the depth of green coloration and progress of unfurling of the leaf blade. The youngest, newly exposed leaves with the unexpanded leaf blade still closely furled and of a pale green

colour were designated *YY*, 'very young'. The somewhat older leaves with the leaf blade partially expanded, but still furled along the margins and of a less pale green colour were designated *Y*, 'young'. The leaves approaching maturity, with the leaf blade just completely expanded and of a darker green than the two previous types but not as dark a green as the darkest green leaves on the plant were designated *YM*, 'young-mature'. Since there was a regular succession of leaves approaching maturity on a growing plant, their relative size and position were also taken into account in assigning types, but these two factors were always treated as subordinate to colour and extent of furledness, for they were often misleading with regard to growth rate, and could not, in themselves, determine the type.

Only one type of leaf was recognized in the second of the main groups. This comprised the full-grown, darkest green leaves on the plant, designated *M*, 'mature'.

The third main group consisted of the leaves that had passed maturity and showed varying degrees of visible senescence, that is yellowing of the leaf. Leaves showing definite yellowing were divided into five types: up to 10% of the total area yellow, *MO*; from 10 to 25%, O_1; 25–50%, O_2; 50–75%, O_3; and from 75 to 100%, O_4. In addition to these five types, all easily recognized by the appearance and extent of true yellowing, there was a less easily recognized type of leaf in which senescence had begun but definite yellowing had not yet appeared. These leaves differed from the truly mature leaves in that they were less glossy and, if they were moved so that the angle of light-reflexion varied, their dark green colour appeared to have a yellowish cast. These leaves were designated *M*(*O*), to indicate that they were mature leaves in the first detectable stage of senescence. It was the different behaviour of the aphids which first suggested there might be a visible difference between *M*(*O*) and true *M* leaves, and the difference was readily detectable, with a little practice, when the leaves were fully turgid and under a slightly hazy sun. If, however, the leaves were flaccid (as they frequently were in the middle of the day during summer) the truly mature leaves lost their glossy appearance and it became impossible to distinguish the two types. Again, the more matt appearance of the *M*(*O*) leaves was difficult to detect in full sunlight. This difficulty could be lessened by shading the leaves, but not entirely eliminated.

Normal senescence of the spindle leaves introduced a colour complication, the red autumnal tints. However, the suitability of the leaves for aphids appeared to depend on the extent of yellowing in the non-red portion of the leaf and the red coloration was, therefore, ignored, the typing of the leaf being based on the extent of yellowing in its non-red portion alone.

During early spring when active growth had begun in the spindle tree, the leaves were all relatively pale. At this stage the *YY* and *Y* leaves could be identified as such by the extent of furledness of the leaf margins. The remaining, fully expanded leaves were at first classified as *YM* since they were of the same pale green colour as the younger leaves on the shoot, yet the behaviour of the aphids and successive measurements of the areas of these leaves showed that many of the apparently *YM*

leaves lower down on the shoots had ceased growth and were in fact *M*. Some of the apparently *YM* leaves were still growing and therefore correctly typed as *YM*. When successive examinations showed that active growth was taking place, it was therefore assumed that the leaves nearest on the shoot to the visibly furled *Y* leaves were true *YM*.

Later in the year when overall growth had slowed down and the mature leaves had become dark green, many leaves that would have been classed as *Y* and *YY* from their colour and furledness, proved nevertheless to have almost ceased growth, and such leaves were, therefore, more truly *YM*. The slowing of growth in such apparently *YY* or *Y* leaves could also be inferred from the fact that the next leaves down the shoot were fully *M*, whereas on growing shoots there was a continuous succession of leaves from *YY* at the apex to *M* lower down. When growth had completely stopped even the smallest leaves became a very dark green and the margins unrolled, and were then correctly classified as *M*.

The leaves on spindle (p. 661) and beet (p. 660) plants making soft growth under greenhouse conditions of high temperature and low illumination never attained as dark a green as leaves on similar plants in normal conditions outdoors. Leaves classed preliminarily as *M* because they were the darkest green leaves on the indoor plants, proved more acceptable to the aphids than outdoor *M* leaves, and began to senesce sooner. They were probably *M*(*O*), incipiently senescent, as soon as they ceased growth, if not before. Visible senescence was recorded in still-growing outdoor sugar-beet plants in the summer (Kennedy & Booth, in press).

More elaborate modification and interpretation of the standard typing system was in general called for with sugar beet than with spindle. Two kinds of outdoor beet culture were employed, corresponding with the two kinds of crop grown commercially, one for seed and the other for roots. In early spring the leaves of the overwintered steckling beets (young plants for the seed crop) were uniformly dark green and therefore all classified as *M*—correctly, as the plants were not growing. When growth began the standard typing system was directly applicable as long as the leaves remained in a rosette. But when an inflorescence was produced, and the flower spike differentiated, the young leaves produced along its stalk developed less and less after the manner of the young leaves on the vegetative part of the plant, until eventually they were developing without having been furled at all, and ceased growth and darkened while still very small. In other words, during the progress toward flowering, the leaves became increasingly part of the flower itself, and no longer part of the complement of normal leaves on the plant. At first sight, these leaves were typed as *YM* since they were unfurled, yet growth-rate measurements showed that they were in fact *Y* or *YM*. Similarly, few of the leaves on the flower spike became as dark a green as the *M* leaves on the vegetative part of the plant, and so were typed at first sight as *YM*, even after measurement showed that growth had stopped, making their correct designation *M*. These flower-spike leaves were very numerous and differed in other ways from the leaves on the vegetative parts of the

plant. It became very difficult to apply the typing system to them, and instead, records were kept of the development of each flower spike as a whole. Three stages were recognized—budding, flowering and seeding—and the true type of individual leaves was estimated accordingly.

The root beets, sown in April, remained in the vegetative phase until lifted in the autumn–winter. In the very early stages of growth, when the darkest green leaves on the plants were recorded as *M* according to the standard typing practice, measurements showed that most of the leaves had not ceased growth so that these leaves were, in fact, *YM*. Once fully mature leaves had appeared and active growth was taking place, the typing system proved to be quite accurate. In the autumn all the leaves gradually became a uniform dark green and were therefore classified as *M*—correctly, since little or no growth was going on.

Results

Myzus persicae *and* Aphis fabae *on pot plants in the greenhouse*

Sugar beets. All the figures here refer to the total populations of aphids, that is the weight or number of all the aphids present on the leaves, including apterous adults, winged nymphs and wingless nymphs.

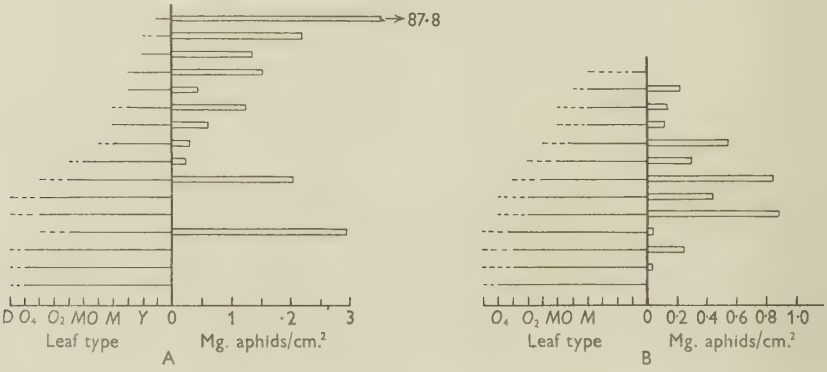

Fig. 1. Leaf-by-leaf distribution of *M. persicae* (by weight) on two vegetative pot sugar beets: A, in active growth; B, making little growth. The leaves are shown in order of their growth, with the newest at the top and the oldest at the bottom. The age-type of each leaf at the time of the aphid counts is indicated by the length of the horizontal line to the left of the vertical axis, and the type it had reached 3 days later is indicated by the broken extension of the line. The aphid density on each leaf is indicated by the length of the horizontal block to the right of the vertical axis.

The distribution of *M. persicae*, by weight of aphids/sq.cm. of leaf under-surface, on a pot beet with very actively growing young leaves but with few early senescent leaves, is shown in Fig. 1 A. The aphids were extremely dense on the youngest leaf, thinned out as the leaves became mature, but were dense again on two leaves which

senesced from O_1 to O_3–O_4 in the 3 days following the counts; no aphids were present on the five initially O_3–O_4 leaves which died during those 3 days. For comparison, Fig. 1 B shows the distribution of *M. persicae* on a pot beet making very little growth but with a number of actively senescing M–O_2 leaves. Here it was the latter, older leaves which carried the greatest density of aphids. These two plants, if considered together so as to make up the complete range of leaf ages, growth rates and senescence rates, provide a two-peaked curve of aphid density: high on actively growing leaves, low on maturing and mature leaves, high again on incipiently but actively senescing leaves and low again on leaves approaching death.

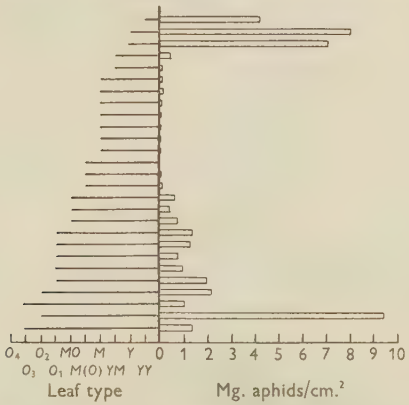

Fig. 2. Leaf-by-leaf distribution of *M. persicae* (by weight) on a pot-bound vegetative sugar beet slowly growing and senescing. Constructed as Fig. 1.

Such a complete curve, with both peaks well developed, was not often observed on one pot plant, but did occur on some rather stunted, pot-bound beets which were slowly putting out new leaves at the heart and slowly senescing among the outer leaves, but bore also a large number of mature, static leaves. The leaf-by-leaf distribution of *M. persicae* on one representative of these plants is shown in Fig. 2. The two density peaks were very clear here, separated by a whole series of middle leaves, in types YM to $M(O)$, which were almost devoid of aphids. Clearly bi-modal curves were also found with *A. fabae* on some pot beets which were also rather stunted and pot-bound, although healthier than the above *M. persicae*-infested beets. These *A. fabae* infestations are illustrated in Fig. 6 of Ibbotson & Kennedy (1950, p. 688).

Occasionally, heavy infestations of *M. persicae* and *A. fabae* developed simultaneously on the same plants, which permitted a direct comparison of the distributions of the two aphids in relation to leaf age. Fig. 3 gives the leaf-by-leaf densities (numbers of aphids/sq.cm. of leaf) of the two species on a large healthy pot beet which was actively growing and senescing at the same time, yet bore a good complement of mature leaves. The same counts are consolidated in Fig. 4A to show the

mean density on each leaf type. Both *M. persicae* and *A. fabae* reached their greatest density on the young leaves, became very scarce on the *YM* and inner *M* leaves, were more numerous again on the outer *M* and the *M*(*O*) leaves and scarcer again on the oldest (O_2–O_3) leaves. But the peak densities of the two species did not occur in quite the same places, the first and second peaks being farther apart for

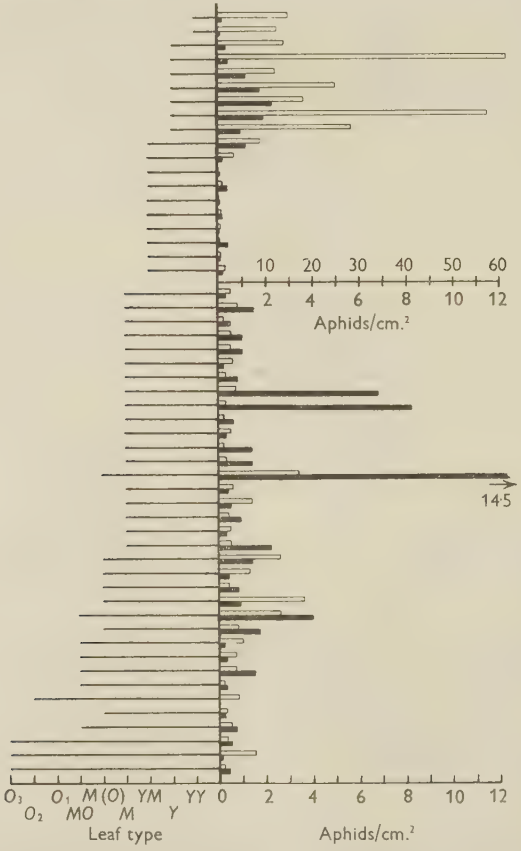

Fig. 3. Leaf-by-leaf distribution (by numbers) of *M. persicae* (open blocks) and *A. fabae* (solid blocks) jointly infesting a large, actively growing and senescing pot beet. Note change of scale where the mature leaves begin. Constructed as Fig. 1.

M. persicae than for *A. fabae* (Fig. 3), and relatively fewer of the *A. fabae* than of the *M. persicae* extended to the youngest and oldest leaves (Figs. 3 and 4A).

The combined results of counts on seven smaller and slightly less vigorous pot beets are shown in Fig. 4B, in terms of the mean number of aphids per whole leaf (not unit area of leaf) of each type. Making no allowance for the unequal size of leaves of course makes the populations on the small, young leaves appear less heavy

than they were relative to the populations on larger, older leaves. The two-peaked shape of the density distribution curves of *M. persicae*, and less markedly of *A. fabae*, which was visible on the plants, is therefore obscured in the figure, although still discernible in the paucity of aphids on the *M* leaves as compared with *YM* and *M(O)*. But the purpose of Fig. 4B is rather to show the contrast between the two aphid species. Here in Fig. 4B, as in 4A, relatively more of the *M. persicae* than of the *A. fabae* were found on the youngest and oldest leaves: the *A. fabae* were more concentrated on to the middle-aged, *YM–MO* leaves.

When the range of leaf-ages present on a plant was incomplete, the distribution curve of the aphids was correspondingly incomplete. If young leaves, or senescent leaves, or middle-aged leaves, were absent, there was only one peak instead of two in the aphid density curve. An asymmetrical curve attributable to the paucity of

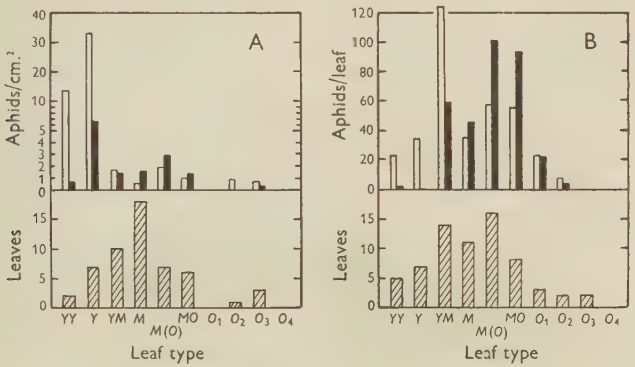

Fig. 4. Distribution (by numbers) of *M. persicae* (open blocks) and *A. fabae* (solid blocks) jointly infesting pot sugar beets. Data from individual leaves consolidated to show the mean density of aphids on leaves of each age-type. A, the same plant and aphids as shown in Fig. 3; B, seven similar but smaller plants.

young leaves has been described above (Fig. 1B), when only the second peak was well developed. The complementary situation where senescent leaves were lacking so that only the first density peak was present, occurred in seedlings. Fig. 5A shows the density distribution of *A. fabae* typical of that found on a number of six-leaved seedling beets. There were no senescent leaves, and the cotyledons were the nearest to true *M* leaves available; the peak density occurred on the other so-called *M* leaves which were in fact not only smaller but also softer and paler than *M* leaves on full-grown plants.

At the opposite extreme of the life-span of the plants, when they were running to seed, single-peaked distributions again occurred, apparently because, although both growing parts (flower spikes) and senescent leaves were present, there were no true *M* leaves between them to create a trough in the middle of the aphid-density curve. The counts shown in Fig. 5B–D were from some pot-beet plants kept in a green-

house which was too warm in relation to the rather poor lighting (due to insect-proof wire gauze walls). There the plants made sickly growth and soon bolted, producing tall, thin flower stalks bearing a series of pale leaves separated by long internodes. The *M* leaves were so called because they were the darkest and glossiest

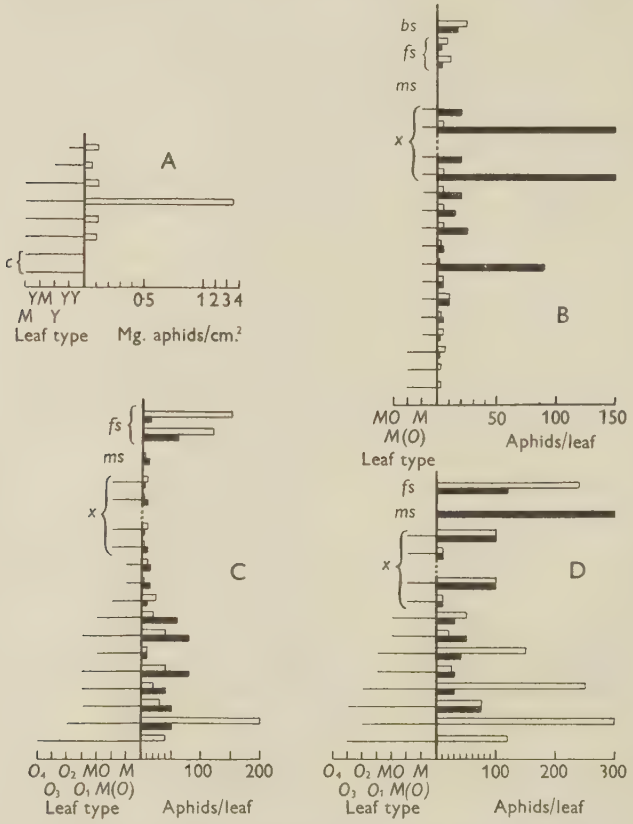

Fig. 5. A, leaf-by-leaf distribution (by weight) of *M. persicae* on a seedling pot sugar beet; *c*, cotyledons. B–D, leaf-by-leaf distributions (by numbers) of *M. persicae* (open blocks) and *A. fabae* (solid blocks) on three flowering pot sugar beets; *bs*, budding flower spike; *fs*, flowering spike; *ms*, main stalk. The region *X*, including the break in the vertical axis, indicates a series of ten or more leaves below the inflorescence, all these leaves being of the same age-type and bearing aphids in numbers ranging between the two extremes shown. Constructed as Fig. 1.

leaves present, but they were softer and paler than the robust *M* leaves present before the plants were put in the screened house. The plants became overrun by both *A. fabae* and *M. persicae*, which were counted on four plants at successive stages of ageing as judged by the setting of seed and the paling of the leaves. The

first three of these plants, arranged in that order, are shown in Fig. 5 B–D. The aphid density on the main stalks and flower spikes is given as the number of aphids (adults and nymphs) per 6 in. (15 cm.) length, to make the unit counting area roughly equivalent to that of most of the leaves and so give a rough indication of relative densities.

The infestations were fairly well dispersed over the plants, neither kind of aphid being absent from a range of middle-aged leaves. But on not too far advanced plants, like those in Fig. 5 B–D, there was still evidence of a trough in the density curve of *M. persicae*, with more aphids on the only developing parts (budding and flowering spikes) and again on the latter senescent leaves, than on the middle-aged, *M–MO* leaves. Something similar is perhaps discernible among the *A. fabae* on plant C, but not on B or D where the *A. fabae* were concentrated in a single, if irregular, density 'hump' among the middle-aged leaves. On more advanced plants, where there were fewer *A. fabae*, they were even more restricted to the middle range of leaves present, now *MO–O*$_2$. The ability of *M. persicae* to thrive on younger and older parts of the plant than *A. fabae*, but of *A. fabae* to thrive on more mature parts, is again evident from the fact that, on plants such as B, C and D, the *A. fabae* usually equalled or outnumbered the *M. persicae* on the *M–O*$_1$ leaves and main stalks, whereas the *M. persicae* outnumbered the *A. fabae* on older leaves and on the budding and flowering spikes. Rough counts on one of the more advanced plants, with seed-setting well under way and no leaves left younger than type *MO*, gave the figures in Table 2. Thus, whether we compare populations on different parts of one plant, or on different whole plants, we find *M. persicae* persisting for longer than *A. fabae* as the leaves senesce, as in Fig. 4A, B. *M. persicae* populations also persisted longer than *A. fabae* on flower spikes as the seed set.

TABLE 2. *Approximate numbers of aphids on different parts of a seeding beet plant*

	M. persicae	*A. fabae*
Flower spikes (per 6 in.)	250	1
Main stalk (per 6 in.)	50	1
MO–O$_2$ leaves (per leaf)	50–200	0–6
O$_3$–*O*$_4$ leaves (per leaf)	20–70	0

Spindle trees. Few counts were made on pot spindle plants because they were not so readily accessible to wandering aphids as the beet plants, and did not habitually carry a wide range of leaf types in a continuous series on single shoots. *A. fabae* colonies did develop preferentially on the younger leaves of growing shoots, sometimes giving marked aphid-density gradients like that shown in Fig. 6, and new colonies appeared on new growth provoked by pruning when the mature leaves on the rest of the plant remained free of aphids. But the greenhouse spindle trees, grown in winter and so in relatively poor light, tended to be rather 'soft', and when they were kept well-watered, *A. fabae* infestations starting on young leaves often

remained *in situ* as the leaves matured, although the rate of reproduction appeared to decline. Diffuse *A. fabae* infestations sometimes developed *de novo* on such plants when all the leaves were apparently *M*. This never happened with *M. persicae*, which was even more restricted to young and senescent leaves, by comparison with *A. fabae*, on spindle than on beet. When the spindle shoots were growing a few *M. persicae* were commonly, and dense clusters of them were occasionally, found on the terminal *YY* leaves, but not lower down on *Y*, *YM* or *M* leaves, where *A. fabae* were found. On senescent shoots, *A. fabae* occurred commonly on *M*–O_2 leaves but disappeared from the O_3–O_4 leaves, whereas *M. persicae* were not uncommonly found on the latter but did not become established on leaves in earlier stages of senescence.

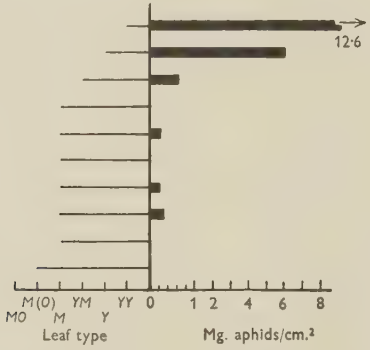

Fig. 6. Leaf-by-leaf distribution (by weight) of *A. fabae* on a growing pot spindle shoot. Constructed as Fig. 1.

Aphis fabae *on outdoor plants*

Sugar beets. Steckling sugar beets were planted out at the foot of spindle trees in several places in the garden in the winter of 1947–8. Small colonies of aptera-mothered black aphids appeared on some of these plants toward the end of April and at the beginning of May, 1948, before any alatae had colonized them. The colonies were found only on beets close to *A. fabae*-infested spindle trees and not near to uninfested trees. On 26 April, when the first beet colonies appeared, ants were seen in active attendance on the aphids on both spindle and beet. Where ants were fewer, the colonies on beets appeared later, when the adjacent spindle infestations were visibly dispersing, not only by the taking-off of alatae (spring migrants), but also by the wandering of adult apterae. These last were seen on 3 May crawling over all the leaves and stems of the infested spindles, over the adjacent soil and over the sugar-beet plants. It was inferred that the beet colonies were in fact being founded, with or without the help of ants, by these apterae from spindle, in spite of the fact that such apterae are not conventionally included in the category of 'alienicolae' because they are not usually found on summer hosts. This appears to be an ecological category

rather than a biological one, depending on the fact that summer hosts are usually too distant from winter hosts for apterae to reach them, so that only the alatae have an opportunity of colonizing them, thereby earning the title of alienicolae. The point of immediate interest, however, is that the new colonies were formed only on the *YY* sugar-beet leaves, not on more mature ones. There were negligible numbers of senescent leaves on the beets at this time.

Detailed counts were made of the distributions of aphids on vegetative ('root crop') sugar beets on several dates in the summers of 1948 and 1949, and a few counts were also made on flowering ('seed crop') beets. These infestations consisted of colonies of all ages from small groups of young nymphs clustered round a lone mother, through big, long-established colonies containing aphids of all ages and forms, many of which were visibly parasitized, to scattered remnants of colonies disappearing as a result of parasite and predator action or the departure of the aphids. Looked at as a whole the infestations sometimes appeared to be distributed almost at random among the leaves of different ages. But, when full particulars of the size and composition of the colonies were recorded, it became clear that the distribution of colonies of any one age was not random, but correlated with leaf age. In analysing and consolidating the figures for presentation here two classes of aphids are considered: the numbers of adults which were accompanied by young nymphs but no late-instar nymphs, and the numbers of aggregates (two or more aphids in close proximity) containing late-instar nymphs. Adults with young nymphs only would not have been *in situ* for more than a few days so that their distribution should reflect the relative suitability of the leaves as nearly as possible according to their ages as recorded at the time of the counts. At the same time, adults unaccompanied by young nymphs, and young nymphs abandoned by their mothers, are excluded from this first class, so as to ensure that the leaves concerned were suitable enough for adults to stay reproducing upon them. The great majority of the total aphids on the plant fell into the second class, presented not as numbers of individuals but as numbers of aggregates or colonies. Most of these groups were larger, and some were far larger, than those in the first class, and the presence of older nymphs and a proportion of visibly parasitized aphids showed that such colonies had been longer established. Omitting single adults or nymphs altogether, from both classes, sharpens the contrast between the classes, since such isolated aphids were usually in transition, dispersing from leaves becoming unsuitable and not yet settled down. Settled aphids were closely aggregated, owing to the strong gregariousness of *A. fabae* (Ibbotson & Kennedy, in press). Even so, the system of dividing the aphid infestation does not, of course, give two entirely distinct classes, just because of the aphids' gregariousness. For example, groups including adults and young nymphs which were certainly new when found on *YY* leaves, were sometimes, nevertheless, classed as old colonies because late-instar nymphs were also present, although these had obviously not been born *in situ*, but had wandered there and aggregated with the other aphids.

The classified results of three sets of counts made at 3-weekly intervals on the same plot of beet in 1948 are shown in Fig. 7 together with the frequency distribution of the leaves on the plants (their 'leaf-age composition'). The figures have all been converted to percentages to facilitate visual comparison of successive distributions. The adults in all these 1948 infestations were almost entirely apterous, and the very rare alatae found are omitted here. Considering first the distribution of the adults which were accompanied by young nymphs, as the most sensitive indicator of current leaf suitability, the histograms for all three dates show two density peaks, one on *YY* and one on $M(O)$–O_3 leaves, with very few such aphids on *Y*–*M* leaves. The density on the *YY* leaves was of course relatively greater than Fig. 7 shows, for

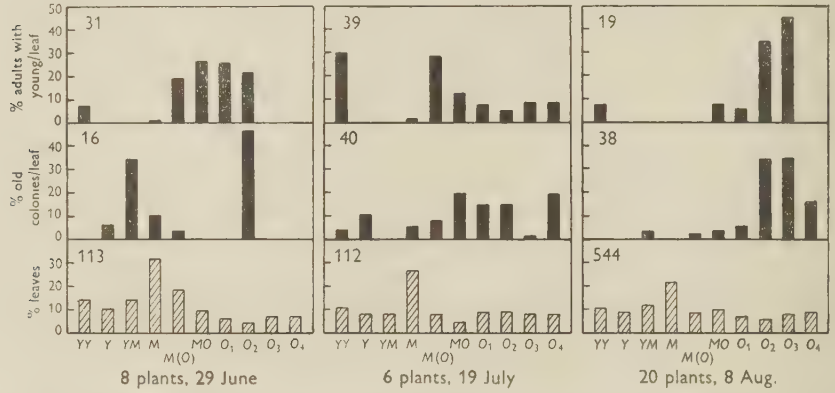

Fig. 7. Distributions of *A. fabae* apterae alienicolae and the colonies founded by them, on an outdoor plot of vegetative ('root crop') sugar beets on three successive dates in 1948. The mean numbers of adults with young nymphs (top row), and of old-established colonies (middle row), per leaf of each age-type, are here expressed as percentages of the sums of the means per leaf on all leaf types. The percentages of leaves of each age type, out of the total leaves present on the plants, are shown in the bottom row. The total numbers of adults with young, old colonies and leaves present, are shown in the corner of each histogram.

no allowance is made for the smaller size of these leaves. The old colonies were concentrated on to older leaves, first *YM* and then *MO*–O_4, than the new ones. This was to be expected if the old colonies had originally started on leaves which were then at the same stages of development as those now favoured by the new colonies, but had remained on the leaves as they passed on to less suitable stages (cf. Ibbotson & Kennedy, 1950). The June–August 1948 distributions thus followed the pattern expected from the counts on indoor pot beets, where also the colonies were all founded by apterae produced on the plants.

The pattern was rather different in July 1949, when the colonies recorded were all founded by winged migrants. The distribution of alatae accompanied by young nymphs on a set of beet plants, which had been retarded as seedlings in pots before

planting out and had hardly begun to bear any senescent leaves at the time of the counts, did show two peaks, on *YY* and *M(O)* leaves (Fig. 8). But the 'trough' between them was less marked than in 1948, for in 1949 the *YY–YM* leaves also bore quite a number of recently settled mothers. Two successive sets of counts on another plot of vegetative beets which had been sown in the open soil and were more advanced, with leaves of all ages up to O_4 present, showed the same kind of difference from 1948, but even more developed (Fig. 9). The recently settled alatae were about equally dense on all leaves from *YY* to *M(O)* on 7 July, and then declined on the O_1–O_4 leaves: there was no sign of a median trough in their density. There was a suggestion of a trough, but no more, on the *YM–M* leaves on 13 July and then a marked trough on O_1–O_2 followed by an odd high peak on O_3 (all due to one single leaf, the only O_3 leaf present at the time) and a sharp decline again on O_4.

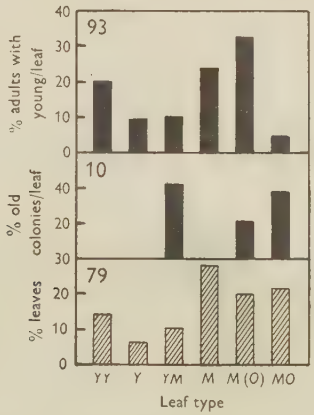

Fig. 8. Distribution of *A. fabae* alatae alienicolae and the colonies founded by them on six outdoor vegetative ('root crop') sugar beets on 7 July 1949. Constructed as Fig. 7.

What other differences were there between 1948 and 1949, which might be connected with the different distributions of aphids on the leaves of different ages? The difference in the form of the aphids concerned, apterous in 1948 and alate in 1949, may have been a contributory factor because there is some evidence, to be published later, that alatae are not as averse as apterae to settling on mature leaves by comparison with young ones. There is, however, sufficient evidence that summer alatae do also prefer young and senescent leaves as against truly mature ones on both spindle and beet plants (Kennedy & Booth, in press), and the main reason for the difference between 1948 and 1949 distributions lies probably in the different conditions of the plants, for the weather of the two summers was very different.

In 1948, cool, wet, spring-like weather continued until the day of the second count, 19 July. There was then a hot, dry spell, but from 1 August it was wet and cool again not only until the last aphid count was taken on 8 August but on until 16 August

when the leaf count used in conjunction with the 8 August aphid counts was made. Reference to Fig. 7 shows that although the proportion of *M* leaves declined somewhat between 29 June and 19 July and again between 19 July and 16 August it remained outstandingly high throughout, and this contrasts with the situation in 1949 (Figs. 8 and 9). A large number of *M* leaves, relative to younger and older leaves, suggests that on reaching the *M* condition the leaves were remaining in it longer than in the other conditions, *YY–YM* and *M(O)–O*$_4$. The *M* leaves present in the spring-like weather of the summer of 1948 thus appear to have been more static and so more truly *M*, in the sense of neither growing nor senescing. If, as seems probable, *M* leaves are leaves at the height of their photosynthetic powers, the proportion of such leaves borne by a plant may be some measure of the vegetative vigour of the plant, by comparison with other plants bearing leaves of similar number and size.

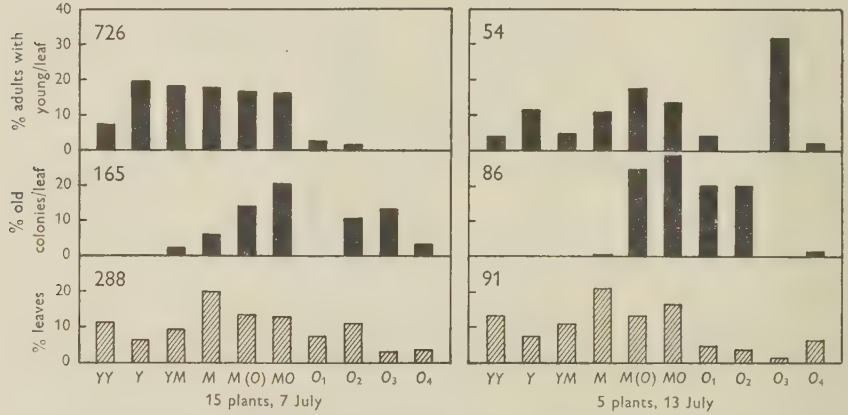

Fig. 9. Distributions of *A. fabae* alatae alienicolae and the colonies founded by them on an outdoor plot of vegetative ('root crop') sugar beets on two successive dates in 1949. Constructed as Fig. 7.

The proportion of such leaves reflects both the absolute number of leaves in the *M* condition and the time leaves remain in that condition, and thus provides a kind of integrated measure of the plant's photosynthetic capacity, if that be the main function of *M* leaves. Perhaps sugar-beet plants are more healthy and vigorous in a certain sense (which may not be the sense of most interest to the agriculturist desiring a high sugar yield in the root) in spring-like rather than in fine summer weather, in this part of England. However that may be, it is noticeable that when there was a high proportion of *M* leaves indicating a pronounced static period in the life of each leaf, that was when the aphid distribution became two-peaked, with maturing and mature leaves clear of aphids. This was apparent also in the counts on pot beets, above.

The summer of 1949 was fine with an exceptionally hot dry spell covering the

period of the two counts in July. *M* leaves were not as numerous relative to younger and older leaves, as they were in 1948. And on 7 July 1949, $M(O)$–O_2 leaves were more numerous relative to *YY–YM* than in 1948. This suggests the occurrence of more active growth and senescence in 1949 than 1948, and relatively more senescence than growth during 7–13 July 1949. The drop in the proportion of O_1–O_3 leaves and the jump in that of O_4's, by 13 July, confirmed the rapid senescence during the preceding days. Thus the leaves were not remaining *M* for so long in 1949 as in 1948, and in that sense were less truly *M*. If visible senescence was setting in sooner in the leaves, it is likely that the invisible process which must precede visible senescence was under way while the leaves were yet *M* or even younger: that is, physiological senescence was setting in before growth had quite stopped. The existence of such 'invisible senescence' was confirmed in other cases (see p. 674). It is therefore suggested that it was the absence of true *M* leaves owing to the onset of invisible senescence before growth stopped, which was responsible for the aphids spreading almost equally over the leaves of all ages from *Y–MO* in 1949, instead of showing a density trough in the middle of this range as in 1948.

There was a marked shift of the second aphid density peak toward older leaves between 19 July and 8 August 1948, and a similar if less marked shift toward older leaves between 7 and 13 July 1949. The total population of aphids fell heavily between 19 July and 8 August 1948, the main loss occurring during the 'heat-wave' from 19 to 31 July when the plants wilted, the aphids became restless and many adults walked off the plants altogether. Thus between 19 July and 8 August 1948 the total number of adult apterae fell from an average of 31·5 to 7·5 per plant and from 1·7 to 0·3 per leaf; the number of apterae accompanied by young nymphs fell from 6·5 to 1·0 per plant and from 0·4 to 0·03 per leaf; and the number of old colonies fell from 6·7 to 1·9 per plant and from 0·4 to 0·07 per leaf. During the great emigration of apterous mothers from the plants it may be assumed that those that wandered off the leaf on which they were born, tended to wander off the plant altogether, instead of moving on to a more suitable leaf to found a colony there. It seems likely that the aphids which did not wander off the leaf on which they were born, and formed a small proportion of those producing new colonies in the period prior to the 19 July counts, would thus come to form a large proportion of those forming the new colonies recorded at the next count on 8 August after the heat wave. The new colonies would come to simulate the distribution of old colonies, as Fig. 7 shows they did, and would thus cease to reflect the true relative suitability of the leaves on the plant at that time.

Rather similar considerations seem applicable to the shift of new colonies to older beet leaves between 7 and 13 July 1949. These colonies were not being formed by apterae on the same plants, but by winged migrants from elsewhere which were starting colonies simultaneously on nearby spindle leaves (pp. 670–1). The number of such migrants found on the plants was considerable in June but declined markedly in July, especially during the very hot period covered by the counts. At that time

there were not only few new arrivals, but many of those that had arrived and begun to bear young on the spindles and beets, disappeared again. Between 7 and 13 July the total number of alatae fell from an average of 54·5 to 10·2 per plant and from 2·0 to 0·6 per leaf; and the number of alatae with young nymphs fell from 48·4 to 10·8 per plant and from 2·8 to 0·6 per leaf. There is thus reason to suppose that the average age of the new colonies may have been greater on 13 July than on 7 July, which would result in their being concentrated on older leaves. These considerations bring out the difficulty of judging the relative suitability of the leaves from counts representing a cross-section at one time, when previous events are not fully known and when, in particular, it cannot be assumed that the adults founding new colonies have had equal access to all the leaves. The whole question is a complex one, and the possibly important effects of changes in the rate of senescence and in the water content of the leaves, cannot be assessed on the available data.

Counts made on outdoor *flowering* beet in 1948 (apterous mothers) and in 1949 (alate mothers) are shown in Fig. 10. The figures are not given as numbers of aphids or colonies per leaf, for the leaf-type distribution was not recorded owing to the difficulty of assigning the large numbers of smaller leaves on the side shoots to types comparable with those distinguished among the crown leaves on vegetative plants (see p. 655). These small, flower-shoot leaves matured quickly and seldom bore any aphids, which were found either on the large basal or main-stalk leaves, or among the flowers themselves. It will be seen that the distributions of aphids were two-peaked, if the flower spikes are included as growing parts comparable with young leaves. But the so-called *M* leaves here, as on the indoor flowering beets, were paler and less glossy than true *M* leaves on vegetative plants, and often carried the peak population density. More advanced plants bearing open flowers, setting seed and many late-senescent leaves, bore fewer aphids than plants bearing flower buds and a higher proportion of *M* and early senescent leaves. The aphids were fewer on the flower spikes as well as on the leaves of the more advanced plants, and even leaves in the same apparent type were, on the whole, less heavily infested on the more than on the less advanced plants.

Spindle trees. As on the indoor spindle trees, clear gradients of aphid density along a succession of leaves of different ages on single shoots, were seldom seen on outdoor spindles. In general, the susceptibility of leaves of different ages could be compared only by examining a number of shoots, or even whole plants, at different stages of development. The available evidence concerns the distribution of colonies founded by fundatrices, by spring and summer (viviparous) migrants and by autumn (gynoparous) migrants.

In the spring, colonies founded by fundatrices hatching from overwintered eggs developed on leaves which were, of course, young at the time. As the shoots elongated the aphids were found to have left the now full-grown, lower leaves they had first infested and to be feeding on the newly proliferated, still partially furled leaves, often on their morphologically upper surfaces. Later generations, however,

tended to remain more on the same leaves as they matured, leaving the younger apical leaves clear, until the aphids took wing or were eliminated by predators and parasites. Such leaves often became markedly curled, raising the question of whether this physiological response of the plant to continual feeding by aphids might

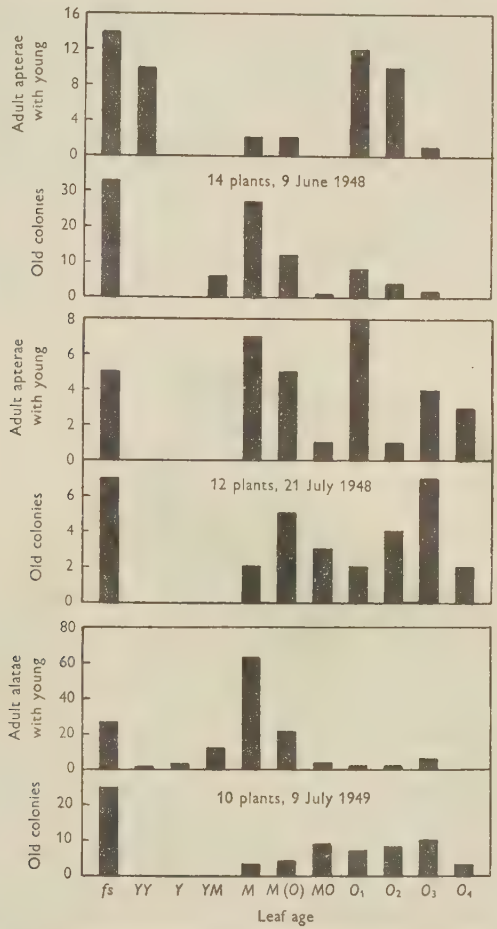

Fig. 10. Distributions of *A. fabae* apterae (in 1948) and alatae (in 1949) alienicolae and the colonies founded by them, on outdoor flowering ('seed crop') sugar beets; *fs*, flower spikes. Expressed simply as total numbers on all leaves of each age-type.

at the same time render the leaves more suitable for the aphids than normal *M* leaves. When growth ceased on shoots bearing heavy infestations, the aphids soon became restless, and were seen wandering all over, and off, the plant, but many of them settled and reproduced for a while on the stems bearing the leaves they had left, feeding through the thin green bark.

After a static period, growth was renewed on a few shoots in May and June, which permitted a comparison of infestations on young and mature leaves. The distribution of what may be called 'spring colonies', that is colonies with apterous mothers and derived ultimately from fundatrices, was recorded on a number of the still-growing shoots in mid-June 1948. The combined results (Fig. 11) showed some concentration of the colonies on to *Y–YM* as against *YY* and *M* leaves, in spite of the fact that *M* leaves were by far the most numerous and the younger leaves had not long existed. Toward the end of May 1949, when the spring colonies were fast disappearing from the great bulk of spindle shoots which were now static, large flourishing colonies persisted for several weeks longer on a few still-growing distinctly sappy shoots which had sprung up in the secondary growth phase from the bases of some of the spindle trees.

At various times in May, June and early July, 1948 and 1949, when the spring colonies were disappearing or had quite gone from the spindles, winged migrants

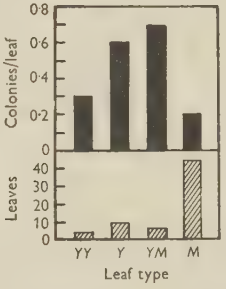

Fig. 11. Distribution of colonies founded by *A. fabae* fundatrigeniae on outdoor spindle shoots in June 1948, expressed as numbers of colonies per leaf of each age-type.

appeared there and deposited nymphs. Most of the winged mothers disappeared again in a few days. These alatae beginning to re-colonize the winter host must have come either from the spring colonies on winter hosts or from early established colonies on summer hosts, and were of course 'alienicolae', strictly speaking. The new colonies were always sparse on the spindles by comparison with their concurrent numbers on summer hosts such as seeding sugar beet, beans, etc. But in all, scores of such small, alata-mothered, summer colonies were seen on spindle leaves in the 2 years. And where actively growing shoots were still present, the great majority of these colonies were found on the partly furled terminal leaves of such shoots, with relatively few on the other, static shoots bearing only robust, mature leaves.

These colonies started by spring and summer migrants usually were very small and failed to complete even one generation. They did not develop into heavy infestations like the spring colonies, or the autumn colonies started by the gynoparous migrants. Predators were partly responsible for this failure, but the condition of

the leaves seemed to have an important influence. Where the colonies died out, the leaves on which they had started were maturing quickly. But where more persistent and softer growth was occurring from the stumps of a number of drastically pruned pot spindles kept outdoors, heavy, many-generationed infestations developed from colonies started by winged migrants in June–early July 1949. And in 1948, heavy infestations developed from colonies started by winged migrants, or by apterae wandering off nearby sugar beets, on a few sappy shoots which sprang up in late August from the bases of some of the spindle trees. No such colonies developed on the other, far more numerous but static spindle shoots. These late-summer colonies persisted in mainly apterous form into the autumn and then, like the colonies on summer hosts, gave rise to winged gynoparae. Most of the gynoparae, on acquiring wings, left the shoots in question, but some of them (or possibly, gynoparae arriving from elsewhere) produced sexual females *in situ*, intermixed on the same leaves with the apterous virginoparous mothers and their apterous and alate

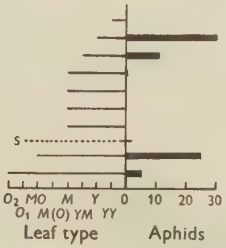

Fig. 12. Leaf-by-leaf distribution (by numbers) of *A. fabae* apterae alienicolae in long-established colonies on a soft, late shoot of an outdoor spindle tree in October 1948. *s*: start of secondary growth. Constructed as Fig. 1.

offspring (cf. Davidson, 1921). In October, these shoots presented an unusually complete range of leaf types, and Fig. 12 shows the two-peaked distribution of the adult apterous virginoparae along one of them. A further opportunity of judging the suitability of senescent spindle leaves for alienicolous summer forms of the aphid, alatae in this case, was created in July 1948, by putting out a pot spindle which had been forced in the greenhouse in the winter, and was now senescent, with leaves ranging from *MO* to O_4. Winged migrants soon formed numerous small colonies on the leaves, while the mature leaves on nearby pot and naturally grown spindles remained clear.

When the outdoor spindle trees were colonized by return migrants (gynoparae and males) in the autumn, there were not usually any growing leaves present. All the leaves were apparently mature or senescent, so that the relative suitability of young and mature leaves for these autumn forms of the aphid could not be judged from normal outdoor plants. However, in autumn 1948 there were two pot spindles in the garden which had put out new leaves in late summer after de-foliation of some shoots by caterpillars (*Yponomeuta* sp.), and therefore bore young growing leaves as

well as the undamaged mature and early-senescent ones on the static shoots. On 26 October, after a few days when *A. fabae* were on the wing, the forty-three young leaves present on these two plants had collected altogether eight gynoparae. All of these were accompanied by newborn nymphs, totalling fifty-eight, and there were no groups of motherless nymphs. The 139 *M–MO* leaves on the same plants had collected a total of six gynoparae, that is less than one-quarter of the number per leaf on the young leaves. Accompanying five of these mothers were a total of twenty-seven newborn nymphs. One gynopara was without nymphs and there were two small groups of young nymphs abandoned by their mothers. Thus the young leaves were apparently more suitable than the mature or early-senescent leaves.

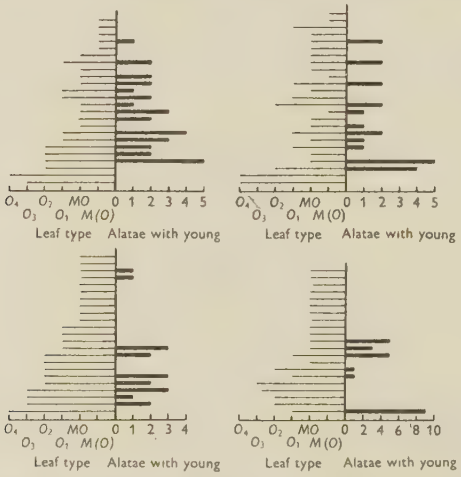

Fig. 13. Leaf-by-leaf distributions (by numbers) of *A. fabae* return migrants (gynoparae and males) with young nymphs, on four outdoor spindle shoots in October 1948. Constructed as Fig. 1.

The relative suitability of mature and senescent leaves for autumn migrants was brought out clearly in counts made on a large spindle tree on 19–20 October 1948. Six of the longest shoots were selected for the counts, at a time when they bore a wide range of leaf types so that in this case individual shoot maps, comparable with the beet maps, can be given. The males, which formed 30% of all the alatae, seemed to be distributed in the same way as the gynoparae at this time. Later, on 12 November, collections were made of all the aphids on several shoots of the same tree, and were found to comprise fifty-three gynoparae, forty-six males, 106 adult oviparae and a large number of nymphal oviparae. 52% of these males and 69% of the adult oviparae were wandering and mating on the stems and some of the latter were laying eggs. 39% of the gynoparae were also wandering on the stems, the remainder being still on the leaves. But on 19–20 October all three forms were confined to the leaves,

together with the nymphal oviparae. The distribution of all those alatae which were accompanied by young nymphs on four shoots, chosen for diversity, are shown in Fig. 13. The combined data for all six shoots, subdivided in various ways, are shown in Fig. 14, as means per leaf of each type. The density of those alatae which were in aggregates with young nymphs showed a pronounced peak on O_1 leaves, and fell away both on more mature, $M(O)$–MO, and older, O_2–O_4, leaves. The number of compact aggregates (consisting mainly of nymphal and adult oviparae, with any number of alatae from none to ten) per leaf, also showed a peak on O_1 leaves, but did not decrease so markedly on O_2–O_3 leaves, presumably because young nymphs are less active than adults (Ibbotson & Kennedy, 1950) and so more liable to be carried over on the leaves where they were born after the leaves have passed their peak suitability. The declining suitability of leaves passing into types O_2–O_4 is evident from the frequency distribution of leaves bearing scattered aphids owing to the break-up of the compact aggregates.

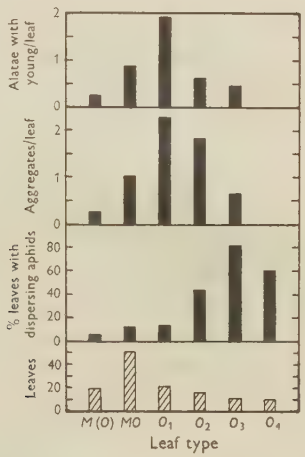

Fig. 14. Distribution of *A. fabae* return migrants and the colonies founded by them on six outdoor spindle shoots in October 1948 (including the four shoots detailed in Fig. 13). Expressed as mean numbers of alatae (gynoparae and males) with young nymphs, and of mean numbers of aggregates of adults-plus-nymphs, per leaf of each age-type; and as percentages of leaves on which the aggregates had broken up. The numbers of leaves of each age-type are shown at the bottom.

One week later than the above counts, the evident correlation between leaf type and aphid density had become even closer. The uninfested (mainly terminal) leaves had remained unchanged in the same types, $M(O)$–MO, as they had been a week before. On the other hand, the $M(O)$–MO leaves bearing aphids (mainly lower down the shoots) had now senesced to MO–O_2. It was therefore the more *actively* senescing leaves that became infested, and not merely the leaves that were at a certain

stage of senescence when first recorded (fuller evidence of the same kind is given in Ibbotson & Kennedy, 1950).

Table 3. *Incidence of colonies being started by autumn migrants on leaves at successive positions along spindle shoots*

	Cambridge, 24. ix. 48		Corley, Warwickshire, 27. ix. 48	
Node number from base of shoot	No. of shoots with leaves at stated nodes	Percentage of nodes with leaves bearing aphids	No. of shoots with leaves at stated nodes	Percentage of nodes with leaves bearing aphids
1	33	45	31	61
2	31	29	28	29
3	29	17	17	6
4–6	34	6	49	4
7–12	20	0	39	3
12–20	12	0	0	—
Total nodes	159	—	164	—

Leaves of so wide a range of ages were found together only on the longest shoots, and it was only on these shoots that a central peak of aphid density, between two low-density regions, was found. On shorter shoots the leaves were more nearly of one age and were on the average further advanced in senescence than the leaves on the long shoots. It was only on the distal parts of the latter, generally speaking, that leaves younger than O_1 and noticeably avoided by the aphids, were common at the time of the counts. Thus the distal leaves on the longest shoots were the last to be overtaken by senescence, while the most proximal leaves on such shoots were generally the first to senesce, simultaneously with other leaves also close to the previous year's wood, but on shorter shoots. Moreover, these proximal leaves, nearest to the old wood regardless of the length of the shoot bearing them, were the first to become colonized by return migrants in the autumn, even before most of them were any further advanced in visible senescence than the rest of the leaves on the plant. The counts summarized in Table 3 were taken in the third week of September 1948, when the recolonization of spindle trees was just beginning—nearly a month earlier than the counts illustrated in Figs. 13 and 14. Most colonies consisted of only one winged mother with a small group of her young, and at this time there was no consistent difference, as far as appearance was concerned, between leaves with and leaves without aphids. But there was a consistent difference between the leaves with and the leaves without aphids with respect to their position along the shoots. Table 3 shows that the proportion of colonized leaves, out of all the leaves at a given position (node number) fell off rapidly on passing outwards from the junction of the shoot with the previous year's wood. This provides definite evidence of what has been postulated elsewhere (p. 667; and Ibbotson & Kennedy, 1950) by the term 'invisible senescence'; that is, of leaves undergoing changes which appear eventually in externally visible colour changes, but which are manifested even before that, by the concentration of aphids on to the leaves concerned.

Discussion

The greater susceptibility to aphid-infestation of growing and senescing leaves, than of static, mature ones, was the recurrent theme running through all the diverse observations: of apterous viviparous *Myzus persicae*, and of apterous and alate, spring, summer and autumn forms of *Aphis fabae*; on both spindle trees and sugar beets, both outdoors and in the greenhouse; and both when the given aphid species or form was colonizing its customary host, and when it was colonizing an unusual host, such as *M. persicae* on spindle, *A. fabae* fundatrigineniae on sugar beet, *A. fabae* alienicolae on spindle, and so on. The same principle held with aphids offered leaves at stages at which they do not normally find them in nature: summer migrants preferred senescent to mature spindle leaves, and autumn migrants preferred growing to mature spindle leaves.

The weight of evidence is very unequal for these different cases, and it is not suggested that aphids of different species, forms and ages, or in different physiological conditions, will distribute themselves in exactly the same way on a given plant. Evidence is given on pp. 657–62 that *M. persicae* and *A. fabae* differ in this respect, and evidence will be given elsewhere that different forms of *A. fabae* differ in this respect also. But it does appear that there is a common, general distribution pattern for all the aphids recorded on the two host plants studied. When plants or shoots bore leaves at all stages of development from very young to dying, the distribution—at any rate of the recently established aphid colonies—took the form of a two-peaked curve: high on the young, low on the maturing and mature, high again on the early senescent and low again on the old senescent or dying leaves. When the range of leaf ages present was incomplete, the aphid distribution could be interpreted as the appropriate portion of the complete curve.

The collection of sufficient data on which to base such conclusions was made possible by the simplicity of the method of gauging leaf age—by external appearance alone. But the anomalies and difficulties to be expected with so crude a method did arise. In particular the leaf type *M*, 'mature', proved to be of variable significance. There was not always a trough in the aphid density curve on leaves so classified. Only true *M* leaves were strongly avoided by *A. fabae*, that is *M* leaves which were a glossy dark green and remained so for some time. But this did not render the leaf-typing system useless, even when the types were recorded only once so that the rate of change of type was not directly known. The frequency distribution of the different leaf types on the whole plant, otherwise termed the 'leaf age-composition' of the plant, threw additional light on the relative suitability of the individual leaves. Thus when *M* leaves constituted an outstandingly high proportion of the total leaves on the plant, it could be inferred that leaves were remaining for some time at that stage once they had grown into it. *M* leaves were then 'true *M*', not only in appearance, but in the sense that they were truly static, neither growing nor senescing. Plants carried such true *M* leaves in two conditions: when vegetatively vigorous, like sugar

beet in spring or spindle in summer, and when pot-bound or otherwise arrested like sugar beet in the winter. Soft, sappy growth of spindle did not seem to produce true *M* leaves. Inasmuch as such sappy leaves would not, to judge by their colour and thickness, make so large a photosynthetic contribution to the plant as true, static *M* spindle leaves do, the sappy leaves may perhaps be likened to the leaves of sugar beet in hot weather, which start senescing as soon as or even before they have finished growing.

It is not likely that the crude mechanical properties of leaves, such as cuticle thickness, play an important part in determining which leaves become colonized by the aphids, since they prefer not only young but also senescent leaves to mature ones, and take readily even to mature leaves in certain conditions. And while reactions to other external properties such as odours probably play some part, there is little doubt that gustatory or other stimuli received after insertion of the mouth parts play a major role. It is therefore through study of the developmental physiology of the plants, with special reference to the phloem sap which is the aphids' food, that we may hope eventually to explain aphid distribution. As a basis for further work on these lines, the present findings suggest that photosynthesis may be in some way the main obstacle to aphids colonizing leaves. The copious excretion of honey-dew suggests that aphids usually receive an excess of sugar in the sap they take up, and that their concentration on to young and senescent leaves may reflect the lower concentration of sugars in the phloem of such leaves, relative to the concentration of nitrogenous substances. The high fecundity of aphids implies a heavy physiological demand for such substances for protein synthesis: in this the aphid resembles the growing parts of the plant on which the aphid prefers to feed. And if growing leaves are places where nitrogenous substances are being mobilized for protein synthesis, the senescent leaves which the aphids also favour are places where such substances are being made available, by 'demobilization' (hydrolysis) of proteins, for translocation by the phloem, and where again, photosynthesis is less active. The aphid does not feed on the growing point itself, we would suggest, partly because it cannot obtain sufficient sap by puncturing separate cells. It requires to 'tap a pipe-line', and can do so only when the phloem sieve tubes have differentiated and begun to function.

How does the aphids' preference for flowering parts of the sugar beet fit into this picture? In describing the distribution of *M. persicae* and *A. fabae* on flowering beets, flower spikes were treated as equivalent to young leaves, since both are developing parts and hence delivery points for nitrogenous materials. Yet as the young leaves approached maturity the *M. persicae* populations thinned out before the *A. fabae* populations, whereas just the opposite occurred on the flower spikes as they fruited: the *A. fabae* thinned out before the *M. persicae*, as on leaves approaching death. From an aphid's point of view it seems that seeding must be likened to senescence rather than to growth: flowering and seeding represent, as it were, a secondary, localized outburst of 'senescence' on the plant. The fact that materials

are being moved out of the senescent leaf but into the flowering spike may be of no moment for the aphids. If they feed on the food-conducting channels of the plant, the *concentration* of nutrients, not the direction of their movement, will determine where the aphids congregate. In the sieve-tubes, there may be a parallel rise and fall of nutrient concentration in senescing leaves and flower spikes, for material stored away in the seeds will be as effectively unavailable to the aphids as when it has been removed altogether from a leaf.

Host specificity. The different distributions of *M. persicae* and *A. fabae* among the leaves of both sugar beet and spindle throw some light on the nature of host specificity. The two peaks of *M. persicae* density were on younger and older leaves than the peaks of *A. fabae*, and *M. persicae* showed a stronger avoidance of mature leaves. The contrast between the two species is illustrated by the idealized curves in Fig. 15. Now *M. persicae* appears to be a more restless aphid than *A. fabae*, although

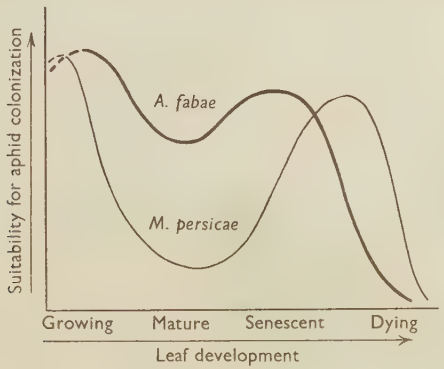

Fig. 15. Idealized curves showing how the relative suitability for colonization by *Aphis fabae*, of leaves at successive stages of development, differs from that for *Myzus persicae*. No meaning attaches to the relative suitability of any one age of leaf for the two aphids; the contrast intended is only between the shapes of the two whole curves. They are based upon the distributions recorded on spindle and sugar beet, to both of which plants *A. fabae* appears to be more specifically adapted than *M. persicae*. The reduction in suitability of the very youngest leaves is conjectural, especially for *M. persicae*.

no quantitative evidence is available on this point. Since the young leaves appear *de novo* and pass on quickly into the next age category, that alone might explain why *A. fabae*, being slower to take advantage of newly available feeding sites, attains relatively smaller numbers than *M. persicae* on the youngest leaves. The shallower trough of *A. fabae* than of *M. persicae* density on mature and near-mature leaves might be explained along similar lines, as due to *A. fabae*'s lesser readiness to move from feeding sites that are becoming unsatisfactory. Yet this kind of difference between the two aphids cannot entirely explain their different distributions, since the second density peak of *M. persicae* comes *later* than that of *A. fabae*. Here, it is

the supposedly less active aphid, *A. fabae*, which abandons the leaves before the more active *M. persicae*, and the same thing happened on the seeding flower spikes of sugar beet. Hence the different shapes of the whole distribution curves of the two aphids probably reflect not so much differences in their general activity, as differences in the leaf conditions which are most suitable for them. A separate study of *A. fabae* on sugar beet (Ibbotson & Kennedy, 1950) showed that the distribution of the whole infestation on a plant depended primarily on selection by the adults present, and that some of these were always on the move, sampling the different leaves.

Although it is difficult to express the matter in precise terms, it would probably be agreed that sugar beet is more specifically *A. fabae*'s host than it is *M. persicae*'s. In other words, although both aphids will colonize a wide range of plants, sugar beet would figure more prominently in a list of *A. fabae*'s than of *M. persicae*'s hosts. This distinction refers, not so much to the commonly heavier infestations of *A. fabae* than of *M. persicae* seen on sugar beet in nature, as to the fact that there are more other plants which seem to equal sugar beet in suitability for *M. persicae*, than there are for *A. fabae*. Turning to the spindle tree, there is no doubt that *A. fabae* is better adapted to it than *M. persicae*, even apart from the question of successful overwintering. On both sugar beet and spindle, *M. persicae* was more closely confined to young and senescent leaves, more strictly avoiding mature ones, than *A. fabae*. On this basis, we may express the rather indefinite notion of host specificity in more concrete biological terms, with the following tentative generalization:

> *The degree of adaptation of a given aphid to a given kind of plant, may be gauged by the extent to which the aphid is able to colonize that plant's leaves* throughout their life; *that is, not only when they are growing and senescing, but also when they are fully functional, in a mature, 'static' condition.*

This hypothesis will be taken further in a subsequent paper elaborating a 'dual discrimination' theory of host selection (Kennedy & Booth, in press).

The host-relations of aphids have been analysed by entomologists primarily in terms of plant taxonomy, the unequal susceptibility of different parts or stages of the plants being recognized, but treated as a subsidiary matter. This may be partly because plant taxonomy and phylogeny are more advanced than plant physiology, but we cannot assume a corresponding one-sidedness in the aphids' acquaintance with plants. Since a real plant is not the fixed unit of taxonomic convention, its condition is as important as its kind, in determining its suitability for the aphid. A given kind of plant may be excluded from the supposed host-range of an aphid, only because the plant is not usually in a suitable condition when the given aphid is on the wing. That is the situation for the alienicolae of *A. fabae* and the spindle tree, and is likely to be so for species, and higher taxonomic groupings, as well as for forms within one species. Thus the spindle tree appears to be a good host for *M. persicae* when making soft, rapid growth in the greenhouse, but not outdoors.

As a plant develops, and according to the way it develops, it becomes something quite different for the aphid. It is noteworthy that in agricultural practice, unlike entomological theory, the varying host-status of a given kind of plant is fully recognized: for instance, in the growing of winter rather than spring beans in order to escape 'black-fly' attack, or in the dictum that a plant can 'grow away' from an insect attack. If such essentially physiological aspects of the host relations of phytophagous insects were given as much scientific attention as the botanical aspects, the possibilities of controlling the pest species might be considerably widened.

Mr H. L. G. Stroyan's indispensable help in separating *Aphis euonymi* from *A. fabae* among the black aphids on *Euonymus* is gratefully acknowledged.

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(*Received* 22 *May* 1950)

THE DISTRIBUTION OF APHID INFESTATION IN RELATION TO LEAF AGE

II. THE PROGRESS OF *APHIS FABAE* SCOP. INFESTATIONS ON SUGAR BEET IN POTS

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(With 7 Text-figures)

Infestations of apterous *Aphis fabae* Scop. on potted sugar beets have been followed in detail for several weeks. The plants were somewhat stunted and their crowns presented an unusually complete series of leaf ages. Records were kept of the changing number and size of the leaves and of their stage of growth. Parallel records were kept of the changing population of aphids on every leaf, and the figures are analysed in various ways to show how suitability for the aphids varied through the life cycle of the leaves.

The leaves were very suitable when young, became unsuitable as they matured, became suitable again just after maturity and then unsuitable again as they senesced. But among leaves at any given stage, those which were growing or senescing rapidly were more suitable than those changing slowly, unless the rate of senescence was very high. The differences of population density on different-aged leaves were due largely to the preferences exercised by the apterous adults. The added effect of differences in the fecundity of these mothers while feeding on different leaves was not excluded, but could not be assessed. It is concluded that the physiological development of the plant as a whole determines, through the growth and senescence among its total complement of leaves, the progress and pattern of its aphid infestation.

Introduction

A number of disconnected observations on the distribution of *Myzus persicae* Sulz. and *Aphis fabae* Scop. on the leaves of two host plants, *Euonymus europaeus* L. and *Beta vulgaris* L. (Kennedy, Ibbotson & Booth, 1950), pointed to the conclusion that young leaves and leaves in the early stages of senescence were, generally speaking, more susceptible to aphid infestation than leaves of other physiological ages. Complementing that work, the distribution of *A. fabae* among the leaves of a few pot sugar-beet plants was studied intensively and continuously for 3 weeks.

The advantage of beet for such a study is that it produces a continuous succession of leaves while the tap root is forming (Watson & Baptiste, 1938). The leaves sprout from the centre of the crown and move outwards towards the periphery as they pass through the stages of maturity and senescence until they eventually die off. The

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simultaneous presence of leaves in all stages of growth and senescence facilitates the comparison of their susceptibility to aphids. The present observations not only confirmed the previous findings but threw some light on their underlying causes.

Material

The plants were grown from seed of a single strain of sugar beet, var. Bush 'E', supplied by British Pedigree Sugar Beet Seed Ltd., Maldon, Essex. The seed was sown outdoors early in 1948, and the plants were lifted during thinning of the outdoor crop on 1 May, re-planted one each to a 4 in. pot, and kept in a cold frame. Their subsequent growth was arrested by comparison with those left in the ground, and they were still small, slow-growing plants when thirty-six of them were brought into the greenhouse at the end of August.

The observations were all taken in a greenhouse in which the temperature varied between 60 and 75° F. (15 and 25° C.), with several hours of sunshine on most days. Under these conditions the plants started a new phase of growth, but this soon ceased, as will be described below. The plants were therefore by no means typical healthy sugar beets, but fulfilled the main requirement of possessing leaves of all ages. It would not have been practicable to make repeated careful counts of aphids, or to avoid disturbing them while doing so, on plants with larger or more numerous leaves. Also, the aphid infestation on these arrested, pot-bound beets was 'zoned' according to leaf age in a more striking manner than is usually to be seen on healthier plants.

The aphids used were derived from a culture of *A. fabae* reared continuously on *Vicia faba* in the greenhouse since October 1946, as described by Kennedy & Booth (1950). Shoots bearing numerous aphids of all ages, the adults apterous and the young both apterous and alate, were cut off the beans, distributed among the thirty-six potted beets and allowed a week to disperse and multiply before the counts began. Five of the least heavily infested plants were then removed to the other end of the bench where it was unlikely that they would be visited by wandering aphids from the main group of plants.

Methods

The plants

The arbitrary scale of leaf types described by Kennedy *et al.* (1950) was employed for classifying the leaves by age. Age types are distinguished on external appearance alone, and indicated by a mnemonic symbol (based on Y = young, M = mature, O = old) as follows:

Type symbol	Brief description
YY	Starting to unfurl
Y	Not quite fully unfurled
YM	Unfurled, but paler than the darkest green leaves present
M	The darkest green and most glossy leaves on the plant

Type symbol	Brief description
$M(O)$	Similar to *M* but less glossy and with a faint yellowish tinge
MO	Definite yellowing of up to 10% of the blade area
O_1	10–25% yellowing
O_2	25–50% yellowing
O_3	50–75% yellowing
O_4	75–100% yellowing

The leaves of a given type varied somewhat from plant to plant, each leaf being classified in relation to others on its plant. Thus the darkest leaf on a plant was classified as *M* even when it was lighter in colour than leaves on other plants which were called $M(O)$.

In addition, the lengths and maximum breadths of the leaves were measured at intervals. A spot of indian ink at the junction of lamina and petiole served as a fixed point from which the longitudinal measurement was always taken. Assuming that the shape of a leaf did not grossly alter, the product of length and breadth was considered an adequate measure of the area for these purely comparative purposes. A reference number was painted in indian ink on the petiole of each leaf. The leaves were measured on 6 and 19 September and 1 October, and their areas are quoted in square centimetres. They were typed at the beginning of each 5-day period of observation, i.e. on 6, 13 and 19 September and also on 1 October.

The aphids

The aphids were separated into the following categories for counting:

Adults. Adult apterae, easily distinguished by their size, shape and elongated cauda.

Alate nymphs. Nymphs with obvious wing buds, that is, alate nymphs in the later instars.

Others. All aphids not in the above categories, that is, all wingless nymphs, and the smaller winged ones that could not be distinguished from them with the naked eye.

Readings were taken of the number of aphids on each leaf every morning during three periods, each of 5 consecutive days, as follows: 6–10, 13–17 and 19–23 September. A final record was taken for all plants on 1 October.

RESULTS

Changes in the plants

The number and average area of leaves in each type on 6 September and 1 October are shown in Table 1, where a general decrease in the size of leaves is evident between those two dates. No two of the plants were quite the same but they fall roughly into two groups. In group 'B', which includes plants III, IV and V, there was an increase in the proportion of *M* leaves, partly through an increase in their actual numbers and

TABLE 1. *Number of leaves and average area (cm.²) of leaves of each type on each plant on 6 September and 1 October*

	Date ...	6 September					1 October				
	Plant group ...	A		B			A		B		
Leaf type	Plant no.	I	II	III	IV	V	I	II	III	IV	V
YY	No. leaves	2	1	2	2	1	—	1	—	—	—
	Av. area	3·7	5·0	4·7	3·9	4·1	—	5·0	—	—	—
Y	No. leaves	—	—	—	—	1	1	—	—	1	—
	Av. area	—	—	—	—	9·4	4·4	—	—	7·5	—
YM	No. leaves	1	1	1	1	1	3	1	1	—	—
	Av. area	25·6	28·1	20·0	26·9	14·0	10·2	13·7	11·2	—	—
M	No. leaves	4	3	3	6	4	3	3	6	7	5
	Av. area	37·0	57·3	38·8	40·7	29·6	25·6	25·0	21·5	28·2	22·0
M(O)	No. leaves	1	1	2	1	2	1	1	—	—	—
	Av. area	40·6	48·8	41·2	96·9	45·3	35·7	30·6	—	—	—
MO	No. leaves	—	—	—	3	1	4	3	—	4	—
	Av. area	—	—	—	89·6	45·0	44·5	58·1	—	41·5	—
O_1	No. leaves	—	—	1	—	1	—	—	—	1	2
	Av. area	—	—	50·0	—	45·6	—	—	—	65·0	33·2
O_2	No. leaves	1	1	2	—	1	—	—	1	—	—
	Av. area	53·1	60·0	55·7	—	57·0	—	—	46·9	—	—
O_3	No. leaves	2	—	1	1	1	—	1	2	—	1
	Av. area	70·3	—	69·4	93·7	43·8	—	60·6	43·7	—	48·1
O_4	No. leaves	1	—	2	—	2	1	1	3	2	1
	Av. area	71·3	—	55·4	—	62·8	41·2	25·8	46·9	54·6	28·1

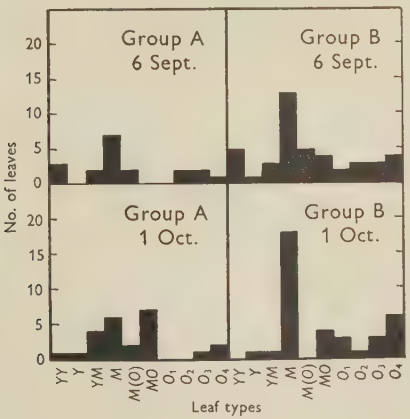

Fig. 1. Frequency distributions of leaf types present on 6 September and 1 October. Group A: plants I and II; group B: plants III, IV and V.

partly through a decrease in the numbers of younger leaves. This did not occur in group 'A', comprising plants I and II. The frequency distribution of leaf types on the plants is consolidated in Fig. 1, which illustrates the difference between the two groups in this respect.

TABLE 2. *Average percentage increases in area of leaves, consolidated into two groups, younger than M, and M or older, from* 6 *to* 19 *September and from* 19 *September to* 1 *October*

	6–19 September				19 September–1 October			
	Younger than *M*		*M* or older		Younger than *M*		*M* or older	
Plant no.	No. of leaves	Increase in area (%)	No. of leaves	Increase in area (%)	No. of leaves	Increase in area (%)	No. of leaves	Increase in area (%)
I	1	80·5	8	14·7	4	20·9	5	0
II	1	64·4	5	6·5	3	32·1	3	1·4
III	1	40·6	11	15·5	2	34·5	6	1·0
IV	1	39·5	11	7·7	6	2·0	7	0
V	2	102·7	11	7·5	1	0	7	0
Totals and means	6	65·5	46	10·4	16	17·9	28	0·5

The changes in growth rate are shown in Table 2, where the data are consolidated. The areas of those leaves which were *Y* and *YM* on 6 September are summed as one unit, and those which were *M* and older, as another. The increase in area, within each of these units, which had occurred by 19 September, is expressed as a percentage of the original area irrespective of the types which the leaves had reached. On 19 September, the leaves are again separated into these two units, according to the types then present, and the same calculations done for the changes they had undergone by 1 October as for the previous period. Table 2 shows that although the growth of leaves which were *M* and older was of the order of 10% during the first fortnight, it was almost *nil* during the second. The growth of young leaves was also much less during the second period than it was during the first, except on plant III. It had ceased on plant V, was vestigial on plant IV, and was still appreciable, though substantially reduced, on plants I and II.

The changes in type of every leaf in the two periods 6–19 September and 19 September–1 October, are illustrated by Fig. 2. The plants are combined into the same groups, A and B, as before. It will be seen that the leaves senesced more rapidly during the second period than they did during the first, particularly in group B.

Evidently there was a considerable change in the state of the plants over the whole period. They had been in the frame outdoors for some time before being brought into the greenhouse and the increase in temperature, together with watering, initiated a spurt of vegetative activity. After 19 September, however, there was a slowing of growth among the young leaves, and a hastening of senescence among

those which had passed maturity. The initial spurt of growth could not be maintained, for the plants were becoming pot-bound.

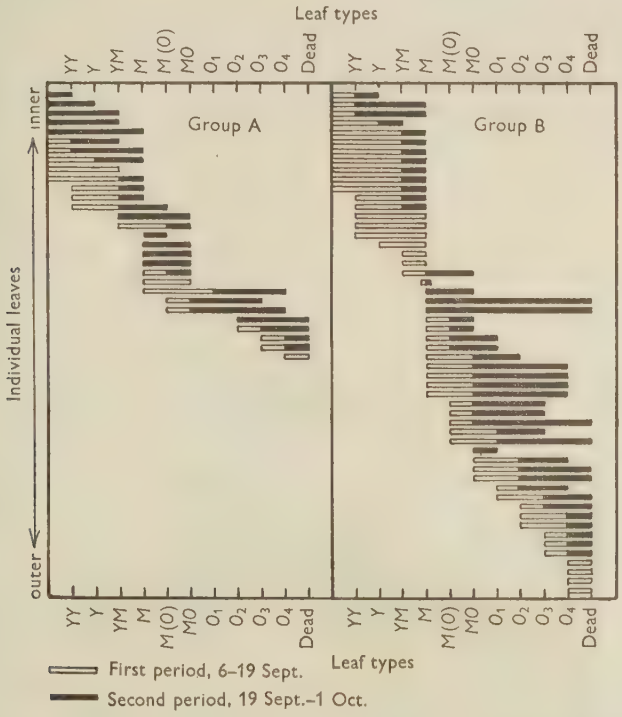

Fig. 2. Growth and senescence of the individual leaves on all the plants.

Progress of aphid populations on the whole plants

The progress of the infestations on the whole plants is given in Fig. 3. The course of events on plant I was similar to that on plant II, and that on plant IV was similar to that on plant V, while events on plant III followed a third, in some ways intermediate, course. These three patterns of infestation development are illustrated in Fig. 3 by plants I, III and IV. Plant I started with a very small population which increased slowly at first and more rapidly towards the end. The number of large alate nymphs reached its maximum at the end of the period, when it was about one-seventeenth of the total young. Plants I and II were the ones in which growth had been only slightly arrested by the end of the observations. Plant IV started with a larger population which increased rapidly until 22 September, when large alate nymphs reached one-quarter of the total young. After this there was a marked decline in the number of aphids, and the proportion of alate nymphs decreased to

one-twelfth. Plants IV and V were the ones in which growth was seriously arrested by the end of the observations. The population on plant III was still rising at the end of the observation period, but had had a previous temporary decline, following a peak around 19 September, the proportion of large alate nymphs increasing and decreasing in parallel but never exceeding one-twentieth of the total young. This plant was intermediate between the others in degree of arrest. The course of the infestation on some typical individual leaves (Fig. 4) shows that the population changes were progressive, without gross discontinuities.

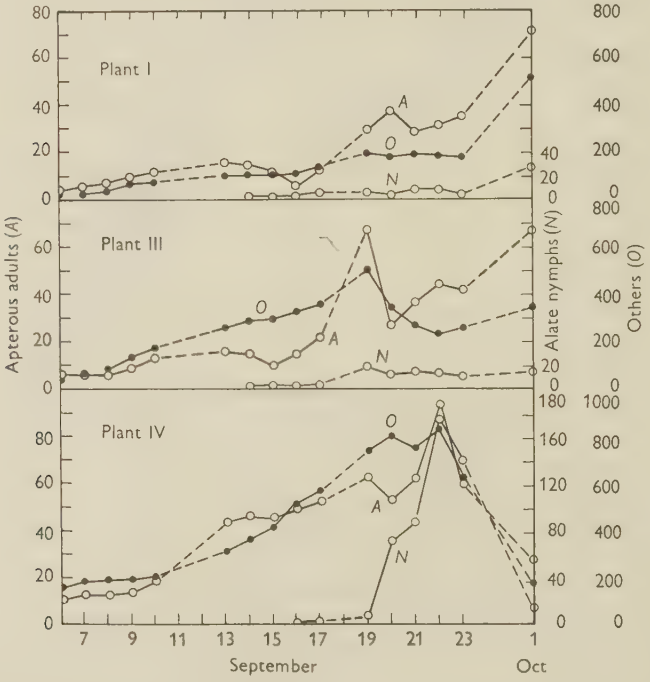

Fig. 3. Progress of the infestations on three representative plants.

Population in relation to leaf age

Total infestations were increasing on all the plants until 19 September. Thus all the plants can be considered as, on the whole, favourable for aphids up till then, and events during this period will be used for the main analysis of the relative susceptibility, to aphids, of different types of leaf. The more heterogeneous events occurring after 19 September will be considered separately.

First period

Distribution of total population. The total numbers of all aphids, apterous and alate, adults and nymphs, on all leaves of each type on 6 and 19 September are shown in Fig. 5. There was a decided peak of numbers on leaves between *MO* and O_2, with small numbers on *Y*, *YM* and *M*, and rather more on *YY*. Beyond O_2 the population declined again, but did not fall as low as it was between *Y* and *M*.

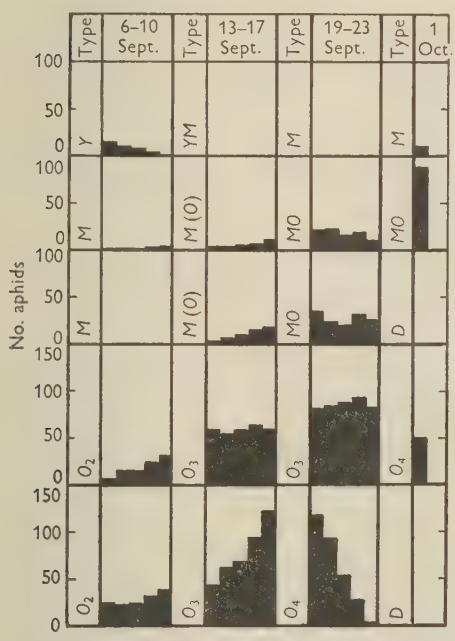

Fig. 4. Progress of infestations on some individual leaves. Symbols refer to the type in which the leaf was classified on the first day of the succeeding 5-day period.

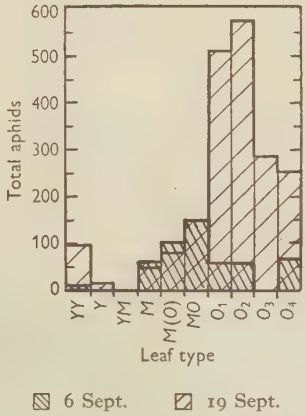

Fig. 5. Distribution according to leaf type of the total population on all plants on 6 and 19 September.

Because the number and size of leaves were by no means equal in the different types, the population density has been calculated per unit area of leaf within each type. The relations which were apparent before this was done (Fig. 5) then become even clearer (Fig. 6) showing that there were real differences of susceptibility between leaves of different ages. Passing from the youngest to the oldest leaves, the infestation density was at first high, fell very low on *YM* and *M*, increased to a maximum on O_1 and then decreased again.

The aphid density shown on the *YY* and *Y* leaves in Fig. 6 is rather misleading. On 19 September, plants III, IV and V had between them three *YY* leaves bearing

a total of ninety-three aphids, and one *Y* leaf bearing fifteen. Since the *YY* leaves were only about half the size of the *Y*'s, it is clear that the aphids were more dense on the *YY* than on the *Y* leaves on these plants. Plants I and II, on the other hand, bore no aphids at all on their two *YY* leaves and one *Y*, and the inclusion of these in the totals used to calculate the numbers of aphids per unit area of leaf type biases the combined results, for all five plants, against the *YY* leaves.

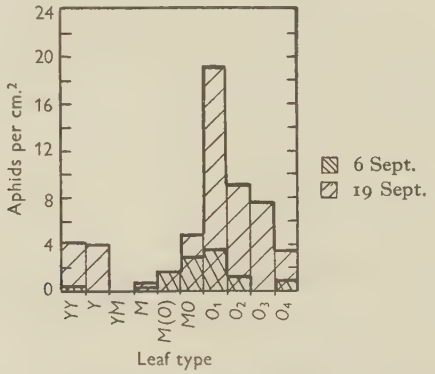

Fig. 6. Density of total population per unit area of each leaf type on all plants on 6 and 19 September.

Since the correlation between leaf type and aphid density was evident even when the unequal number and size of the leaves in different types were ignored, they will continue to be ignored in the subsequent analysis except where otherwise stated.

TABLE 3. *Net increase of population according to leaf age and rate of senescence*

— = no leaf; o = no aphids.

Leaf type	Total increases	Net increases per leaf according to senescence-rate categories 1	2	3	4
YY	42	.	.	.	.
Y	4	.	.	.	.
YM	o	.	.	.	.
M	70	5·5	12·0	24·0	49
M(*O*)	206	—	57·6	190·0	—
MO	265	50·0	—	133·0	—
O_1	160	—	68·0	76·0	—
O_2	156	15·0	80·0	136·0	—
O_3	167	—	3·8	—	—
O_4	2	—	o	—	—
Col. no. ...	i	ii	iii	iv	v

Increases of population. As a first step in analysing how the infestation patterns observed on the plants came into being, the age at which leaves acquired their main bulk of aphids can be estimated. This is found by calculating the net (not percentage)

increases of population on each leaf during successive periods of observation. The increases of population between 6 and 10 September, and between 13 and 17 September, are summed in Table 3. In column i the increases of population are summed for all leaves on all plants, according to leaf type, on the basis of the type classifications done on 6 and 13 September. Most leaves had changed their type by the end of the observation period, and those starting in the same type changed to unequal extents. In columns ii–v, therefore, the leaves of each initial type (as recorded on 6 September) are sub-classified into four categories, according to the amount they had changed by 19 September, thus:

Category 1: remained in the same type.
2: moved on one type.
3: moved on two types.
4: moved on three types.

The mean net increases of aphid population per leaf have been calculated for each senescence-rate category.

It will be seen that the further the leaves advanced in type during the observation period, the greater the increase of population on them, whether the initial type was *M*, *M*(*O*), *MO*, O_1 or O_2. Now, judging from several different methods of assessment (column i of Table 3, column viii of Table 4 and column i of Table 6), these beet leaves appeared to be at their peak of susceptibility to *A. fabae* when in type *MO*. The true peak may be at *M*(*O*), as suggested by column iv of Table 4, since there may be a time lag between a leaf's entering its condition of peak susceptibility, and the visible reflexion of this fact in aphid numbers, during which time the leaf may have passed on into another type. The crudest method of assessment, by the distribution of the whole population, gave a peak at *MO*–O_2 (Figs. 5 and 6). Nevertheless, the rapidly senescing leaves accumulated *more* aphids than slowly senescing ones, even when the former started or finished in relatively unfavourable leaf types such as *M*, or O_3–O_4; and even when the more slowly senescing leaves remained at or near the highly favourable type *MO*. Thus a leaf's suitability for aphids depends not only on the *stage* of senescence it has reached at any given time, but also on the *rate* at which it is senescing.

Aggregation. Evidence of the greater readiness of aphids to remain settled on some leaf types rather than on others, quite apart from whether they reproduced more on some than on others, is available from some special counts done on plants IV and V between 6 and 10 September. Here, the aphids were recorded on a sketch map of the leaf in the actual positions in which they were feeding. They were not settled at random on a leaf but occurred in aggregates, and all the aggregates on the leaf were marked in their relative positions within an outline drawing of each leaf, with a record of the numbers of aphids in each aggregate. For this purpose an aggregate was defined as two or more aphids not more than their own width apart, and separated from any other aphid by at least 2 mm. The positions of solitary individuals, two or more mm. from any other individual, were also marked.

The number of aphids in each aggregate was recorded every morning, and new aggregates were entered on the maps as they formed. This was done only during the first week, 6–10 September, when but few of the young present became adult, so that break-up of aggregates due to maturation of their members did not confuse matters as it did in later weeks.

TABLE 4. *Distribution of permanent and temporary aggregates, and of solitary and aggregated individuals, according to leaf type*

	Aggregates				Individuals			
Leaf type	Permanent	Temporary	Total	Permanent / Temporary	Solitary	Aggregated	Total	Aggregated / Solitary
YY	2	0	2	∝	0	13	13	∝
Y	0	0	0	—	0	0	0	—
YM	0	0	0	—	0	0	0	—
M	3	9	12	0·3	18	65	83	3·6
M(O)	14	8	22	1·8	5	162	167	32·4
MO	11	13	24	0·9	6	291	297	48·5
O_1	3	5	8	0·6	4	68	72	17·0
O_2	3	2	5	1·5	1	33	34	33·0
O_3	(No leaf)	—	—	—	—	—	—	—
O_4	3	4	7	0·8	3	69	72	23·0
Col. no. ...	i	ii	iii	iv	v	vi	vii	viii

For tabulation purposes, the aggregates have been separated into two classes, *permanent* and *temporary*. A permanent aggregate is defined as one which persisted in the same place through the 5 days; a temporary aggregate was one which was formed *de novo*, or which dispersed completely, within the same period. The numbers of permanent and temporary aggregates are shown in columns i and ii of Table 4, where they are segregated according to leaf types as classified on 6 September. The individual aphids were also separated into two classes, those which were solitary, and those which were in aggregates. To the numbers of individuals of each class present on 6 September, have been added any additional ones that appeared on the successive days up to 10 September, to give the totals shown in columns v and vi. Finally, the ratios of permanent to temporary aggregates on each leaf type are shown in column iv, and the ratios of aggregated to solitary individuals in column viii, of Table 4.

It will be seen that no solitary individuals were found on the *YY* leaves, and that the two aggregates there were both permanent. Among the other leaf types, the aggregates were least stable on *M* leaves, and most stable on *M(O)*'s. From *MO* onwards the stability of the aggregates varied. Correspondingly, the individuals were least aggregated on *M* leaves, most aggregated on *MO* leaves and less aggregated again among the older leaves.

Distribution of adults. The relative activity of adults and young was determined by direct counts of the individuals seen moving on the plants on 19–21 September.

Counts of aphids in the three categories, apterous adults, alate nymphs and 'others', were made at 09.30, 12.30, 14.30 and 16.30 hr. The average number of each category moving on each plant at one reading is expressed in Table 5 as a percentage of the average total population of aphids of that category on the plant. The proportions of adults and alate nymphs moving at any one time were more than ten times as great as the proportions of moving 'others'. Among these moving 'others', large apterous nymphs were counted separately from smaller ones, and found to average over 50% of the total. Since the large apterous nymphs comprised at most 25% of the total 'others' on the plants it follows that the older nymphs were more active than the younger ones. The proportions of moving alate nymphs varied from about the same as, to many times more than, the proportion of 'others' moving and was, on the average, nearer to the proportion of adult apterae moving. Adult alatae were hardly ever seen on the plants so that all of them must have moved off the plant within a few hours of the final moult.

TABLE 5. *Mean percentages of adult apterae, alate nymphs and 'others' observed moving on each plant*

Plant no.	Apterae	Others	Alate nymphs
I	2·98	0·28	6·60
II	2·10	0·11	0·00
III	12·80	0·63	8·39
IV	3·20	0·15	0·17
V	9·27	0·70	5·83
Means	6·06	0·37	4·14

TABLE 6. *Distribution of apterous adults according to leaf age and rate of senescence*

— = no leaf; o = no adult aphids.

Leaf type	Total adults on leaves of each type	Total adults per leaf, according to senescence-rate categories			
		1	2	3	4
YY	43	.	.	.	.
Y	2	.	.	.	.
YM	5	.	.	.	.
M	41	2·8	3·6	6·0	4·0
M(O)	120	—	22·0	33·0	—
MO	202	40·0	—	65·3	—
O_1	162	—	10·0	56·0	—
O_2	76	o	6·0	44·0	—
O_3	95	—	2·8	—	—
O_4	14	—	2·8	—	—
Col. no. ...	i	ii	iii	iv	v

Since the apterous adults moved about more than the other categories of aphid on the plants, they may be presumed to have most thoroughly sampled the available range of leaf types. If so, their distribution should be the most sensitive indicator of the feeding preferences of aphids as between different leaves. The daily counts of

the adults have been summed for each leaf type during each of the periods 6–10 and 13–17 September. The totals for the two periods, segregated according to the types in which the leaves were classified first on 6 September and again on 13 September, are given in Table 6, column i. In columns ii–v, the distribution of the adults has been further sub-classified in terms of the rates at which the leaves changed their type between 6 and 19 September, as was done for the net increases in population (p. 689).

The general pattern of adult distribution was the same as that shown by the total population distribution, and by the increases of population. The peak infestation was on the *MO* leaves; and rapidly senescing leaves were more heavily infested than those senescing slowly, providing further evidence (see p. 689) that a leaf's senescence rate, as well as the stage of senescence it has reached, affects its susceptibility to aphids.

Second period

Fig. 7 shows the population distribution according to leaf type for all plants on 1 October. Group A contains plants I and II and group B plants IV and V. The condition of plant III appeared to be intermediate at this time so its aphid population is shown separately, by dotted lines, in the group B part of the figure.

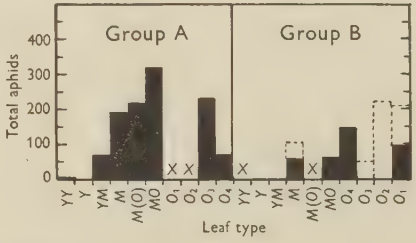

Fig. 7. Distribution according to leaf type of the total population on all plants on 1 October. Group A: plants I and II; group B: plants IV and V, with plant III shown separately by the broken lines. *XX*, leaf types entirely absent.

On the plants of group A, the peak of the infestation had shifted back from the late senescent leaves, where it was on 19 September (Fig. 5), to the *M*–*M*(*O*)–*MO* leaves. The *M* leaves, which had carried no aphids in the first period, now carried more than the *YY*'s, and the *YM*, *M*, and *M*(*O*) leaves all carried more aphids, in proportion to the numbers on the other leaf types, than they had carried at the end of the first period (Fig. 5).

The group B plants now carried only small numbers of aphids altogether. Growth, it will be recalled (Fig. 2), had become arrested in both groups of plants by the end of the second period, but to a much greater degree in group B than group A. At the same time, a greater proportion of group B than group A leaves were senescing in the second period (Figs. 1 and 2), and the rate of senescence among these group B leaves was now higher than it was at any time in group A.

Analysis of the population increases and adult aptera distributions during this period showed no regular relation between either and the rate of senescence.

Discussion

The aphid population on the beets was clearly zoned among the leaves according to their age, so long as the population was still growing (Figs. 5 and 6). There were a number of aphids on the very young leaves, but comparatively few on the *Y* to *M* types. The bulk of the population was on those leaves which had just passed maturity, and fewer again were on the oldest leaves. The total population on any of these leaves depended on: births *in situ*, immigrations from other leaves, departures to other leaves, or departures off the plant altogether. There is no direct evidence of the relative importance of these factors in determining the unequal populations of leaves, but some indirect evidence can be adduced given the facts that:

(i) Leaves acquired large populations, not through sudden influxes, but by a steady accumulation of aphids over a period of time (Fig. 4).

(ii) Even when the population density was at a maximum, less than 1% of the young but up to 12% of the adults were moving about on the plants (Table 5).

It seems to follow that the bulk of the population on any *YM* or older leaf consisted of aphids which had remained there from birth, and not of immigrants. Thus it was the minority of more mobile apterous adults which mainly determined the distribution of the population, by settling preferentially on certain leaves, and depositing young there. This is consistent with the finding that the bulk of the population, having been on the *MO* and younger leaves on 6 September, was on leaves which were O_1 and older by 19 September (Fig. 5). The leaves which were *M*(*O*) and *MO* on 6 September had changed their type to O_1 or older by 19 September (Fig. 2); that is, the leaves carried most of their aphids with them as the leaves grew older.

With this we may contrast the course of events on the *YY* and *Y* leaves. Although some individuals may have stayed on the *YY*'s as these leaves matured, the bulk of their populations evidently left before they became *YM*. Since the *YY* leaves, *as a type*, nevertheless showed an increasing population, they must have been repeatedly re-infested. Thus, as long as leaves remained *YY* the adult apterae were very inclined to settle on them, but did not remain long enough to leave many young there because the leaves soon passed into the most unacceptable types on the plants.

It follows, from this 'carry-over' of aphids on ageing leaves, that the observed zonation of the whole aphid population among leaf types does not truly reflect either the settling preferences of the adults, or variations in their nutrition affecting their fecundity, or the two combined which we may term the *relative suitability* of leaves for the aphids. A rather better measure of relative suitability can be obtained from the rest of the data.

The increases in population for each leaf type show the combined effect of settling preferences and fecundity differences (Table 3); *M* leaves were the least suitable of

the leaves older than *YM*, while *M*(*O*)'s and *MO*'s were the most suitable. The distribution of adults, taken by themselves, gave a similar picture of relative suitability. Furthermore, the suitability of a leaf evidently increased with the rate at which it was senescing, as shown by the data both on population increase and on adult distribution.

Some indication of the role of settling preferences, as apart from fecundity differences, is available from the data on the relative permanence of aggregates (Table 4). These suggested that the aphids were least restless on the *M*(*O*) and *MO* leaves, quite apart from whether or not they were most fecund there. There is no evidence against the view that the aphids were also most fecund on those leaves, but these observations do not permit any more positive statement to be made. It has been shown experimentally, however (Kennedy & Booth, in press), that the fecundity of adults does tend to be greatest on the leaf types on which they prefer to feed.

To sum up, the data for the first period show that the most suitable leaves were *YY* and *M*(*O*)–O_1. The *Y* leaves were also suitable, but as they quickly became *YM* and thus completely unsuitable, large populations could not develop on them. The suitability of *M* leaves was slightly greater than that of *YM*'s, as some colonies began to develop on them. The greatest absolute increase of population occurred on *M*(*O*) and *MO* leaves. Populations which developed on these leaves tended to remain, but declined as the leaves became older, until the aphids left altogether just before the leaf died. The suitability of *M* and older leaves evidently depended not only on the stage of senescence they had reached, but also on the rate at which they were senescing. Over the range of senescence rates obtaining during this first period, the suitability of leaves increased with their rate of senescence.

Events during the second period, when the infestations were decreasing as a whole on some of the plants, were more confused. But, if examined closely, they are not inconsistent with the conclusions drawn from the first period. On plants I and II the populations did not begin to increase rapidly until well into the second period (Fig. 3), indicating that these plants were as a whole more suitable during the second period than during the first. Their growth was retarded during the second period (Table 2) and, judging by plants IV and V where the slowing of growth had gone even further (Table 2) and there was clear evidence of a concomitant acceleration of senescence (Fig. 2), it is probable that senescence (or the invisible processes responsible for visible senescence) was also accelerated on plants I and II. If so, this may account for their increased suitability for the aphids in the second period as compared with the first.

However, although the course of events on plants I and II during the second period resembled that on plants IV and V during the first period, to some extent, there was a difference here. On plants IV and V during the first period, the population remained static on the *YM*, *M* and *M*(*O*) leaves and almost so on the *MO* leaves, while increasing on the older ones, as shown for all the plants combined in Figs. 5

and 6. Whereas on plants I and II during the second period, the population increased on the *YM*, *M*, *M*(*O*) and *MO* types both absolutely and relatively to the other leaf types (compare Fig. 5, 19 September, with Fig. 7), in spite of the fact that these leaf types were now growing much more slowly than the same types were growing on plants IV and V in the first period (Table 2). Visible senescence was, however, sometimes observed even while leaves were still growing. It must depend on invisible processes within the leaf which to some extent precede their visible expression. Perhaps the invisible processes were further advanced in the *M* and even in the *YM* leaves on plants I and II, and this accounts for their developing fairly large aphid populations, by contrast with the *YM* and *M* leaves on the more completely arrested plants IV and V.

On plants IV and V the populations increased rapidly during the first period, and declined during the second (Fig. 3). These plants were therefore more suitable during the first period than the second. Their greater suitability during the first period may have been due to the fact that they were then senescing more rapidly than plants I and II (Fig. 2). During the second period, the growth of plants IV and V was almost completely arrested (Table 2), and the senescence of leaves older than *M* was proceeding even faster than on plants I and II at any time (Fig. 2). The leaves older than *M* now carried only small populations by comparison with those types during the first period, and their poor suitability may have been due to their senescing *too* rapidly (Fig. 2). Consequently, by the end of the period, there were no *M*(*O*) leaves and four *MO*'s, as against four O_3–O_4 leaves. Whereas on plants I and II there were nine *M*(*O*)–*MO* leaves as against three O_3–O_4 (Table 1). Thus plants IV and V were approaching a completely static condition, that is one in which all the leaves would be virtually *M*'s.

Plant III was intermediate in many respects. Its senescence rate was intermediate during the first period, and the aphid population increased to an intermediate extent. Growth continued during the second period, but by the end of it there was only one leaf younger than *M* left, with six *M*'s, no *M*(*O*)'s, *MO*'s or O_1's, one O_2 and five O_3–O_4's (Table 1). Thus plant III was approaching the static condition of plants IV and V. But the senescence rate was not so high on plant III during the second period as that on plants IV and V, where it appeared to be too high, and leaves older than *M* still carried a fairly large population at the end of the period.

Thus the rise and fall of the aphid infestation on the plant as a whole can be interpreted, in this preliminary way, in the light of the growth and senescence of its individual leaves. The infestation does not depend simply on what we may call the 'leaf-age composition' of the plant, that is on how many leaves of each of our arbitrary age-types are present on the plant. It depends also on how fast the leaves recorded in any given age-type are moving toward maturity or death, and the growth rate and senescence rate of a given plant are not fixedly related. The infestation depends, in short, on the developmental physiology of the whole plant.

The work was done while the first author held a scholarship from the Agricultural Research Council, and his thanks are due to Dr V. B. Wigglesworth for granting him working facilities with the Unit of Insect Physiology.

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(*Received* 15 *March* 1950)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Annual General Meeting of the Association held on Friday, 24 March 1950, in the Imperial College of Science and Technology, London; the President, Mr G. Fox-Wilson, in the Chair.

Formal business was dealt with and the following papers were read and discussed:

1. Some aspects of plant pathology in Australia. By Mr N. T. Flentje.
2. The interaction of strain and host genetic factors in symbiotic nitrogen fixation. By Dr P. S. Nutman.
3. The root surface flora of banana varieties, and disease resistance. By Mr J. L. Harper.
4. Technical aspects of the East African groundnuts organization. By Dr A. H. Bunting.
5. The use of apple rootstocks in outdoor testing of fungicides. By Dr M. H. Moore.
6. The effect of date of application of nitrogen to wheat in presence and absence of eyespot. By Mr G. A. Salt.
7. A striking effect of mineral nutrition on the ecology of a plant community. By Miss A. V. Delap.
8. The soil plate method: a simple way of isolating fungi from soil. By Dr J. H. Warcup.

SOME ASPECTS OF PLANT PATHOLOGY IN AUSTRALIA

By N. T. Flentje, *Waite Agricultural Research Institute, South Australia**

The range from the tropical crops of Queensland to those of the cool temperate climate of Tasmania gives Australia a wide variety of fungus, virus, nematode and physiological disorders of plants. Economically, the diseases of horticultural crops are of great importance, particularly those of grapes, stone fruit and of vegetables grown for canning.

Soil deficiencies of nitrogen, phosphorus and potash are widespread and in the coastal region deficiencies of the minor elements are common. In certain irrigated districts, notably the Murray valley citrus areas, unwise use of irrigation water has led to salt accumulation in the soil.

Until recent years, land, in many areas, was not a limiting factor. Many farmers would therefore plant extra acreage to compensate for anticipated disease losses, rather than carry out control measures. Such practices have resulted in heavy losses following epidemics and have favoured the build-up of residual infection, particularly of soil-borne fungi.

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Organization of plant pathological services

The foundations of the study of plant pathology in Australia were laid by Daniel McAlpine, who was appointed Government Vegetable Pathologist in the Department of Agriculture, Victoria, at the end of the nineteenth century. His successor, C. C. Brittlebank, published a catalogue of Australian fungi, which has been kept up to date and is still widely used.

At present, the basis of the organization of plant pathology in the individual states is the central laboratory which is located in the capital city of each state. These laboratories include relatively few plant pathologists—one or two in some states, up to ten in others—and their work is partly advisory and partly research. In advisory work they have the assistance of the general agricultural advisers, who are not plant pathologists but can give the growers information on the commoner disorders, referring anything unusual to the central laboratory. Research in plant pathology in each state is carried out both by the Government laboratory and by the University, frequently in collaboration.

Individual states have modified this general scheme to meet their special problems. In Queensland the importance of sugar-cane cultivation has necessitated the establishment of special research and advisory facilities for this crop: in South Australia the state phytopathological service is provided by the pathology department of the Waite Institute, which is part of the University of Adelaide. In Victoria and in New South Wales, there are regional agricultural and horticultural research stations carrying out local investigations, but few of these have a resident plant pathologist.

Supplementing the state services, the Federal Government provides phytopathological services administered by the Plant Industry Division of the Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O.). Plant pathologists are stationed at the headquarters at Canberra and at a number of regional stations. These co-operate with the universities and state agricultural departments to carry out inter-state investigations, and the regional laboratories (at Griffith, New South Wales and Merbein, Victoria) undertake local advisory work.

Difficulties of plant pathological work in Australia

An outstanding obstacle to the development of plant pathology in Australia is shortage of staff. In the universities, the lack of men graduates in botany is a matter of grave concern. There are wide possibilities for British plant pathologists in Australia as recruits to the service, or as post-graduate students or as visiting workers on an exchange basis. A second adverse factor is the isolation and lack of professional contacts in many appointments. The contrasts between work in the tropical north and in the temperate south are a further hindrance to co-operation and the free movement of staff between states. Mobility is also frustrated by differences in salary levels and terms of service between adjacent states.

The distances to be covered and the delays in exchanging information are among the causes responsible for many serious gaps in knowledge of even the commoner diseases. These difficulties have been aggravated by limited facilities for publication.

New trends in organization

Following an inter-state conference of plant pathologists arranged at Melbourne in May 1949 by the C.S.I.R.O., it was decided to hold similar conferences regularly. Australian Journals of Scientific and of Agricultural Research are now being published. Other steps in co-ordination now being taken include the establishment of a national fungus culture collection at Canberra, and the improvement of inter-state co-operation in the plant sciences. An integrated service of plant pathology in Australia appears to be arising.

TECHNICAL ASPECTS OF THE EAST AFRICAN GROUNDNUTS ORGANIZATION

By A. H. Bunting, *Overseas Food Corporation*

World food needs and the geographical setting of the scheme

If the physiological requirements for food for the human species are to be met many million acres of new agricultural land, probably over 100 million, must be brought into productivity within the next half century. There are three principal zones of the world in which possible reserves of land and climate of this order exist. These are (1) the podsol zone of the northern parts of the Eurasian land mass, (2) the red earth zone of South America, principally in the Amazon basin, and (3) the red earth zone of east and central Africa.

It is in the last of these zones that the operations of the Overseas Food Corporation in East Africa are concentrated. We are presently concerned with three regions, all lying in Tanganyika; the Kongwa region, in the Central Province; the Urambo region, in the Western Province, and the Southern Province region. The Kongwa region has an annual rainfall of from 20 to 25 in. and lies approximately at 3500 ft. elevation above sea-level; it is 200 miles from the Indian Ocean at the port of Dar-es-Salaam, to which it is connected by railway. The Urambo region, about 200 miles farther to the west, and on the same railway, is at much the same elevation, but the annual rainfall is at least 10 in. higher. The Southern Province areas are from 70 to 150 miles from the sea in a straight line, and although the port facilities available are still somewhat primitive, a railway to the sea was recently opened to traffic. The areas lie at an elevation of 1200–1500 ft. and have a rainfall of about 35 in. per annum.

These rainfall figures must of course be considered in relation to the seasonal distribution of the rain. In all three regions there is a marked rainless season of at least 6 months. At Kongwa, the rains begin in most years in December, and there is a marked rainless gap of a fortnight or so, starting towards the end of January and reflecting, in this approach to an equatorial type of distribution, the low latitude of the region. The Kongwa rains end effectively, again in most years, at the end of April. The rainfall situation at Kongwa, with this distribution and a total below 25 in. average, is clearly marginal, and the priority of development there as against the other areas available was dictated by its advantage in respect of communications. Nevertheless, we have already shown by soil moisture studies that under our system of management, reserves of water can be accumulated in the subsoil at Kongwa. A dry farming system based on such accumulation is, in fact, the objective of research in that region.

At Urambo the onset of the rains comes in late November, and they continue, with something of a gap in late January, until the end of April. At an average precipitation of 35 in. this distribution is not disadvantageous. In the south, although general features are similar to those at Urambo, the general reliability of the rainfall is far higher, owing to the overlapping, in a zone stretching far south into Portuguese East Africa, of the south-east trade and north-eastern monsoon rainfall systems. This region is also somewhat farther from the equator, and less dominated by the typical equatorial bimodal rainfall pattern.

Meteorological studies by the Corporation's Scientific Department are being directed principally to the more detailed investigation of the spatial distribution of rainfall. For this purpose gauges are sited on a grid at 2-mile intervals in all cleared land.

The southern and western regions lie in country typical in many ways of a wide belt of Africa extending through Portuguese East Africa, Tanganyika, Northern Rhodesia, the southern parts of the Congo and Northern Angola, in which the development of much new agricultural land is probably technically possible. It is indeed not too much to say that the operations of the Corporation are gaining the essential experience on which the long-term rational opening up of these lands, which are at present largely unused and for the most part unoccupied for lack of surface-water supplies, can be based.

Vegetation and soil types

In all three regions the areas being developed by the Corporation were formerly almost entirely uninhabited. Botanical evidence, and the signs of former human activity, make it clear that this was not always the case, but time does not allow me to explore that fascinating avenue this afternoon.

The typical natural cover at Kongwa is a dense, thorny, impenetrable, deciduous thicket, characterized by several species of *Commiphora* with *Grewia* and other genera, and in parts with large baobabs (*Adansonia digitata*) and *Acacia* spp., notably *A. spirocarpa*, in rather moister situations. The general height of this formation is from 15 to 20 ft. The blanket of thicket is cut up by considerable flat grassy areas, some of which were formerly the beds of shallow lakes. In the south and west the most important vegetation type consists of variants of *miombo*, that immense, amazingly homogeneous open woodland community, up to 60 ft. high typified by numerous species of the related leguminous genera *Brachystegia* and *Isoberlinia*, which extends over hundreds of thousands of square miles in East and Central Africa, from the Southern Sudan to Southern Rhodesia, and practically from the Indian to the Atlantic Oceans, in the 30–50 in. rainfall belt.

The first stage in the development of an area is the broad mapping of the topography, soils and vegetation, based on ground work and aerial photographs. The object is the determination of the availability of land, the rough limits of the production units, and the general form of the soil conservation system. From the map produced, the clearing operations can be directed, and the engineers can be guided to some extent in the location of unit centres and installations such as workshops. There were formerly no reliable large-scale maps of the areas in which the Corporation is working.

This preliminary survey is accompanied and followed by more detailed work carried out by the Scientific Department, aimed at the recognition of the main soil types and their genetic interrelations. The main soil types of all three regions are now known and their pedology is approximately understood. The main mature soils, throughout, are tropical red earths, but owing to the extreme peneplanation of the terrain more than half of an area may often be covered by soils formed of transported material, in a catenary sequence. These soils may be yellow, grey or black, but their topographic position shows clearly that they are genetically related to the red earths. The essential first problem is the recognition not so much of the characteristic soil types as of the characteristic catenary sequences.

The next stage, the clearing of the standing bush, now follows, after much experiment, a well-defined pattern. After the area has been opened up with machine-cut tracks, the initial felling is accomplished by two tractors pulling between them a length of anchor chain, which simply pulls the trees over and when the ground is moist brings out the heavier parts of the root system as well. This is followed by the piling of the debris. At Kongwa, where development is now complete, this was done on contour lines, so that the piles, which were later burnt, formed the nucleus of the conservation system. In the other regions present practice is to pile in a simpler manner and to burn the piled material, leaving the development of conservation works until later.

The third step is the extraction of the remaining stumps and roots. This is mainly done by drawing a heavily mounted horizontal transverse blade through the soil. Research and operational study is being directed to the improvement of this process.

The removal, by means of large mechanical rakes, of the root and stump debris is now followed at Urambo and in the Southern Province by the development of the first stage of the conservation system. Variable grade contour banks, which may later be transformed into broad terraces, are put up, using a mechanical grader.

Finally the soil surface is levelled by an implement like an immense peg-tooth harrow, constructed of lengths of railway track. This operation is of great importance for the production of level seed-beds.

Up to the present time about 97,000 acres of land have been prepared for agricultural use.

Of these 76,000 acres have been cleared from standing bush, the remainder representing former open grassland. These acreages are distributed as follows: Kongwa, 81,000 acres, of which 21,000 were formerly grassland; Urambo, 14,500 acres; Southern Province, 1000 acres.

The development at Kongwa has now, as I have said, reached its limit for the time being. That at Urambo will be complete by the middle of 1951.

Soil and water conservation

The protection of the land against the very real danger of water erosion is thus an integral part of the clearing system. It can safely be said that all land at Kongwa and in the south, and much of the land at Urambo is safe from the risk of major erosion, although inter-terrace sheet wash is still a problem. Broad-based terraces have not been constructed except in limited areas, on account of the time and expense involved; but the very effective contour banks produced in the clearing will, as time goes on, be developed with the aid of the plough into broad-based terraces, and additional protection will be put in as required.

The risk of wind erosion is small at Kongwa, as our experience of over 3 years there has shown. In the wetter regions, clearing is so conducted as to leave uncleared strips in drainage depressions and along ridge lines, as well as along access roads. These strips, supplemented if necessary by additional planting, should eliminate the wind danger, which has however not declared itself so far. Naturally, in all this work our growing experience guides development, and changes in our operational plans are readily made as needed.

Soil fertility

Soil fertility investigations carried out by the Scientific Department fall into two parts: the laboratory (physical and chemical) characterization of the main types, and the determination, by means of field experiments, of the main plant nutrient requirements of the main crops on the main soil types. This is naturally a very long-term programme, but we have already reached a broad general impression of the fertility status of the soils. Acutely acid soils are rare, the base status being apparently maintained by frequent additions of potash in the past resulting from bush fires. Calcium is, however, occasionally very low, in soils of higher elevation in the wetter areas, but liming does not appear to be needed generally. The most characteristic and general deficiency in all regions is in phosphate, and we have already shown the advantages to be gained from placement of phosphates. Nitrogen is not required at present for groundnuts, whose nodules, formed without any need of inoculation, appear to supply all needs in this respect. We have shown the value of nitrogen dressings for non-leguminous crops, but have yet to find the best way to present fertilizer nitrogen without running severe risk of seed-bed damage.

Fertilizer experiments are running at present at the rate of 150 per year, but as we are studying the fertilizer needs of six major crops on at least eight main soil types, it will be realized that this number will have to be at least doubled in order to make adequate progress.

Although most of our soils are from the beginning very low in organic matter (2% loss on ignition is an exceptionally high value on upland soils) we are interested in the study and analysis of the effect of organic manures and of the incorporation of crop residues as part of rotational sequences. Our colleagues of the Empire Cotton Growing Corporation at Ukiriguru, in the Lake Province of Tanganyika, have recently found that certain dramatic long-term residual effects of organic manures can be explained by the effect of the material on phosphate status. We are following up this line on one of our own stations.

Experimental crop studies

The soil fertility work just mentioned is for the most part conducted on experimental sites scattered throughout the production areas so as to lie on all the main soil types at a range of situations. The experimental work of the Scientific Department on crops, crop husbandry

and crop protection has, however, required the establishment of three full-scale mechanized experimental farms, of 700–800 acres, one in each of the three regions, central, western and southern. These farms have their own European agricultural staff together with junior specialists in soils, plant pathology and entomology as we manage to recruit them. There are several aspects of the work of these farms. Field-scale experimentation on cultural practices compares the effects on yield and on the cost of production of minimal cultivations with those of more orthodox European style methods. We have reason to suspect that annual ploughing is uneconomic and probably unnecessary under some of our conditions at least, and that vigorous weeding soon after emergence is of far more importance than later cultivations. These hypotheses are at present being tested on a semi-operational scale on so-called pilot experiments, in which the individual plots are 10 acres and factorial arrangements are used, although so far without replication.

The main work of the farms, however, is the study of crops and crop varieties on large- and small-scale observation plots as well as in formal experiments and variety trials. Planting dates, plant population, row distances, spacings and similar questions all come under review. The establishment of pasture, fodder and soil conservation grasses is also investigated. Plant breeding work, from which great advantages are to be expected, is however held up for lack of a senior plant breeder.

The principal crops now being grown on the farms, out of more than thirty tested, are groundnuts, sunflower, sorghum, maize, safflower, castor bean, soya bean and niger oil, and we are constantly on the look out for new types of these and other crops in all tropical and sub-tropical countries. We see no reason at present to doubt that from among these crops satisfactory rotations can be built up. Experiments to study rotations are under way but must, of course, continue for a number of years before precise answers can be given. In the meantime we are conducting a number of 2-year experiments to measure the immediate residual effects which may follow the incorporation of bulky crops and crop residues.

Crop mechanization

Work on this subject is among the many important responsibilities of the Operational Research Unit, which is organized and directed independently of the Scientific Department. Roughly speaking, the task of the Scientific Department is to discover how best to produce the crops of choice; the operational research workers then find out how to apply the scientific results on the very large scale. The need for such work was shown in the first season. In that year at Kongwa, experiments in the production fields gave an average yield, in the presence of phosphate, of over 800 lb. per acre of groundnut kernels. On the experimental farm at Kongwa the figure was over 1000 lb. of kernels. Yet in the production fields as a whole only 400–500 lb. were brought in. The difference was largely due to mechanization difficulties, principally in harvesting and seed-bed preparation. These are clearly matters for operational research, and there are many others.

The heart of the mechanization problem lies in recognizing that farm machinery has, in many instances, to be very precisely and yet flexibly adapted to local climatic, soil and biological conditions. Equipment which succeeds in one area must not be expected to work in another, and in Tanganyika we must expect to find as time goes on that we have to modify or redesign much of the machinery which we use at present. The commercial firms who are supplying us are fully alive to this and they are giving us very valuable help in the development work.

Another special soil management problem in this field arises from the natural tendency of the tropical red earths to compact on drying.

PLANT PROTECTION

The entomological and plant pathological work in the Scientific Department is combined under one senior officer. This facilitates a unified ecological attack on all plant-protection problems. This is made the more advisable since our major problem in this field, the rosette virus disease of groundnuts, involves both insect vector studies and disease investigations in the field. Streak disease of maize, also an insect-borne virus disease, is potentially serious, but so far the acreage of maize planted has been small. Other plant diseases, although in total fairly numerous, are of very low incidence. Attention is being concentrated on diseases which are known or suspected to be soil-borne, since they are the most likely to build up to dangerous proportions more or less independently of climatic conditions. Of such diseases, the chief in Tanganyika at present is crown rot of groundnuts, a seedling disease with which a number of fungi are associated, the main one being an *Aspergillus* of the *niger* group. Recent evidence from Northern Rhodesia indicates that the fungus *Sclerotium rolfsii* may in some areas and under some conditions become a serious danger in a very short time, so this is under close watch.

Entomological work on rosette disease which is, by the way, confined to the wetter areas and is unknown at Kongwa, has been developing along three lines: insect vector studies, particularly in relation to dry season survival, studies of field spread and incidence of the disease, and work on the control of secondary aphis multiplication by means of systemic insecticides. The field work of this programme has been concentrated in Western Tanganyika. We are greatly aided in work on rosette by Dr H. H. Storey, who is conducting valuable work on plant-vector relationships at Amani.

We have not been able to show that the vector passes the dry season in the natural bush surrounding the Urambo area, and indeed groundnuts planted in the bush do not develop symptoms of the disease even in a year of very high incidence. There is no relation between infection and distance from the bush margin. It seems likely that infected vectors enter the Urambo area from native lands 13 and more miles to the east, being carried on the prevailing winds. They enter in quite small numbers—a few hundreds to the acre only, and the progress of the disease turns on the extent of the secondary spread. The aphids may be heavily attacked by various predators, which in a season favourable to the predators may keep the disease incidence at a very low level or may even eliminate secondary spread altogether. When the predators are not effective late planting may show infection up to 100%.

Insect cage studies have shown that certain systemic insecticides are capable, at very low rates of application, of controlling secondary spread of aphids. This work is now being extended to field trials, using very low volume applications, and studies of residual toxicity are being arranged.

The entomologist has carried out detailed studies of pollination in sunflowers and has shown that at Kongwa and Urambo, even in a dry season in which far fewer florets were produced than in a normal year, natural pollination, mainly by wild honey bees, was inadequate. This season we are studying the number of bees required to secure effective pollination, and future work may well involve the development of large-scale bee production to provide sufficient numbers of bees at flowering time. It is worth stating that our surveys indicate that the wild bee population, before clearing, would have been far from adequate in numbers at flowering time to pollinate the crop effectively.

The pentatomid insect *Calidea dregei*, formerly known as a locally extremely serious stainer of cotton, last season caused major losses in sunflowers, principally by penetrating the hull of the developing seed and feeding on the contents. It has also given trouble in maturing sorghum. So far, we have not found any commercial insecticide which promises any hope of effective control, and life-history studies are not yet far enough advanced to suggest any biological means of control. We do not, naturally, know yet the variations in intensity of infestation by this insect from year to year, and work is continuing on all possible lines of

attack. We have assembled a considerable armoury of the newer insecticides for this and similar work.

Calidea is our only example up to date of an unimportant natural inhabitant of the bush becoming a major pest after clearing.

In all aspects of the plant-protection work the essential background is perpetual vigilance, both in our own areas and in native farms, even at a considerable distance.

Organizational aspects

The Scientific Department of the Corporation has now been in existence for just over 3 years. It has an European staff of just under thirty, of whom only a handful have advanced research qualifications. These specialists, who are attached to the departmental headquarters at Kongwa, have assistants on the staffs of the three experimental stations. At headquarters, we maintain central chemical and biological laboratories (now moving into a suitable building after 2 years in tents), a library and a statistical laboratory. A small pathological laboratory is being established at Urambo. A vital part of the department's work has been the training of senior African assistants, who now number about thirty. Some are already developing considerable proficiency in routine work, both in the field and in the laboratory, and add very materially to our resources.

The scientific department has the free right of scientific publication and of collaboration with other scientists. In particular, we have developed close collaboration with the East African Agricultural and Forestry Research Organization under Dr Keen at Nairobi, with the East African Agricultural Research Institute under Dr Storey at Amani, and with the Tanganyika Department of Agriculture. These three bodies are represented by their directors on the East African Scientific Committee of the Corporation which reviews the annual programme of research of the Department.

The results of the work in the 1947–8 season were published last year by the Overseas Food Corporation (1949)* and the results for 1948–9 are in active preparation for similar presentation.

Conclusion

If I were to sum up my conclusions after three years, they would reflect a very high degree of confidence in the technical future of the Scheme. In general, we have, I think, learnt in broad outline how the clearing can best be done, and the development of improved equipment is in some instances complete, in others far advanced. On the agricultural side, although there will always be the hazards of tropical weather, we have so far met no problems which are new, in the sense that they are of a type which has not been encountered and successfully overcome elsewhere in the past. The enthusiasm of the staff for the tasks ahead is of a high order, even though the tasks themselves are more numerous and more difficult than were at first expected. The creation of a new system of agriculture, suited to the conditions of our areas, is not an easy task, nor one that can be accomplished overnight. But, for the greater part of the land with which we are concerned, if not for all, we are certain that it can be done.

* Overseas Food Corporation (1949). *Annual report and accounts.* H.M.S.O.

REVIEWS

Laboratory Methods for Work with Plant and Soil Nematodes. Technical Bulletin no. 2, by Dr T. GOODEY. Pp. 20 + 10 plates. London: His Majesty's Stationery Office. 1949. 9*d.*

This bulletin is a concise compendium of practical methods for use in the study of free-living and plant parasitic nematodes. Workers in this field owe a further debt of gratitude to Dr Goodey for collecting together, in handy form, techniques which he and his colleagues have proved in practice to be eminently satisfactory, and many of which they have developed. The information is simply and logically arranged, beginning with the collection of nematodes from plant tissues and from the soil. It then gives simple directions for making temporary mounts for immediate microscopic examination. Suitable techniques for producing permanent mounts both of isolated eelworms and eelworms stained *in situ* in leaves, stems and roots are clearly described. Finally the bulletin sets forth a number of miscellaneous methods including the culture of nematodes on artificial media; the introduction of eelworm infection into potato tubers and a number of very useful hints on drawing and measuring. The photographic illustrations of apparatus add greatly to the value of the text. I know from personal experience of many of these methods how useful they are as an aid to both teaching and research and the bulletin will be welcomed as an essential laboratory manual by the growing number of nematologists. It may, in addition, stimulate interest among pure zoologists and encourage them to take up the study of this very important group of invertebrates.

L. R. JOHNSON

The Establishment of Vegetation on Industrial Waste Land. By R. O. WHYTE and J. W. B. SISAM. Pp. 66. Aberystwyth: Commonwealth Agricultural Bureau Joint Publication no. 14. 1949. 10*s.*

The present conflict between agriculture, industry and amenity for the land of Britain gives added emphasis to the need for the reclamation of industrial waste areas. This publication reviews the extent of the problem both in this country and elsewhere and deals with the types of waste land, methods of survey, the factors determining choice of treatment, natural colonization by plants, and artificial establishments of herbage or trees.

The physical factors considered include stability, erosion, drought, waterlogging and temperature. The effects of the last-named may be exemplified from Pennsylvania, where it was found that the high surface temperature of the bare mineral aggregate was the principal hazard to seedling establishment, and was overcome by temporary shading with brushwood. Chemical factors discussed include liberation of sulphuric acid from sulphides, persistence of toxic constituents beneath a shallow layer of weathered material and deficiencies of essential mineral nutrients—mainly nitrogen, phosphorus and often lime. The discussion clearly shows the need for expert handling of the problems.

The economic return and value of projects for the reclaiming of industrial waste land are difficult to evaluate. The effects are indirect as well as direct: the influence of depressing, ugly and derelict areas in a town on the productive capacity, vigour and health of its inhabitants should not be underestimated. In spite of these difficulties of assessment one could wish for more details of the economic aspects of the problem as they concern *this country*, and especially of the cost per acre for the restoration of some of the diverse examples cited. These would be especially welcome in view of the fact that the implementing of many desirable projects depends on the reaction of a committee to an estimate of probable capital expenditure and maintenance charges.

The 60 photographs are an outstanding feature of the book. They show vividly both the

desolation of the waste lands caused by past and—be it noted—present industrial activities and the great scope that exists for correction or avoidance of what is still a national disgrace.

The book is well produced, clearly printed and free from errors. The omission of a subject index is, however, regrettable in a volume containing such a wealth of specialized and previously unpublished information. It can be heartily recommended to all concerned with the face of the land and should appeal particularly to ecologists, advisory officers and planners.

E. J. HEWITT

La Variation. By É. GUYENOT. Pp. 630. Paris: G. Doin et Cie. 1950. 1500 fr.

This is a companion work to *L'Hérédité* by the same author, already reviewed in this *Journal*. Having in the earlier volume dealt with the mechanism of heredity, he now turns to the materials which it manipulates. The first part comprises a fairly detailed listing of the known genes in invertebrates, vertebrates and plants. This leads naturally to a discussion of the process of mutation and of polyploidy, polysomy and chromosomal aberrations. Finally, he deals rather summarily with population genetics and continuous variation. The latter chapters are the least satisfactory part of the whole book. Of the 12 pages devoted to continuous variation (compared with 30 pages on mutations in the mouse) at least three-quarters are consumed by a discussion of what is meant by a distribution.

Considered as a whole, it tries too much. Rather too detailed for a general text-book, it is still not sufficiently complete to replace the specialized monographs such as, for instance, Gruneberg's *Genetics of the Mouse*. It gives one or two tantalizing glimpses of the books that might be written and for which there is a definite need as, for instance, an attempt to integrate present knowledge on mammalian genetics. The historical sections, which introduce each new topic, are of great interest. The references are copious and the book closes with a bibliography of some 2000 references.

ALAN ROBERTSON

Genetics, Palaeontology and Evolution. Edited by GLENN L. JEPSON, GEORGE GAYLORD SIMPSON and ERNST MAYR. Pp. xiv+774. Princeton: University Press. 1949. $6 (39*s.*).

Evolution is, or should be, the central point towards which all biological science converges. This was well understood during the time of Darwin and the following decades. But in the present century the demands of an ever-growing specialization are such that few workers in any one field find it possible to keep abreast with developments in others. This has been especially true of genetics, which in its amazingly rapid stride has, on the one hand, outstripped the attempts of non-specialists to follow its development, and on the other hand has been so fully occupied with its own discoveries, new methods and theories that it has tended to disregard as irrelevant the discrepancies between its own, Neo-Darwinian interpretation of evolution and the evolutionary theories of palaeontologists, anatomists, and taxonomists. The last decade has seen a re-awakening of the spirit of synthesis between the diverging viewpoints, exemplified by a number of publications by leading biologists of various branches. The present book is the outcome of an unusual type of co-operation. At the 1941 meeting of the Geological Society of America a group of geneticists, palaeontologists and systematists started informal discussions on evolution. This was the germ for the Committee on Common Problems of Genetics, Palaeontology and Systematics formed in 1943 to bring together scientists from the three fields for the pooling of information, the discussion of common problems, and the development of new lines of research. After several years of co-operation through meetings, letters and the distribution of printed bulletins, a final symposium was arranged in Princeton in 1947; on this occasion the group was enlarged by additional specialists in several fields of geology and biology. A symposial volume was prepared from the contributions of a number of participants, each an acknowledged authority in his field.

After a foreword by Glenn L. Jepson, the stage is set in a first chapter on 'Time in Earth History' by A. Knopf. The second part 'Viewpoints on Evolution' maps the various paths of

approach: genetical (Curt Stern on 'Gene and Character'; Warren P. Spencer on gene homologies in *Drosophila*), palaeontological (D. M. S. Watson on fossil vertebrates), anatomical and morphological (D. Dwight David and Theodor Just). The third part on 'Evolutionary Trends' starts with a discussion on 'Time Series and Trends in Animal Evolution' by A. Sherwood Romer, followed by special chapters on dipnoi (T. Stanley Westoll), rhinoceroses (H. Elmor Wood, II), and angiosperms (Ralph W. Chaney). The fourth part on 'Evolutionary Rates' consists of two general chapters by G. G. Simpson on animals and by G. L. Stebbins Jr. on plants, and one special chapter on taeniodonts by Bryan Patterson. In the fifth part on 'Speciation', E. Mayr deals with 'Speciation and Systematics', D. Lack with ecological isolation, E. B. Ford with allopatric speciation in a moth, J. A. Moore with speciation in *Rana*, W. Hovanitz with hybridization in butterflies, and H. L. Mason with the fossil and recent history of *Pinus remorata*. In Part VI on 'Adaption' Sewall Wright outlines the general theory, while E. H. Colbert illustrates it by examples from Palaeozoology. Part VII on 'Human Evolution' consists of a lecture in which J. B. S. Haldane discusses problems of the biological past and future of mankind. Finally, in 'Summation', H. J. Muller draws the many threads together into a closely knit exposition of evolutionary theory as it emerges from this synthesis of scientific disciplines.

The high competence which appears in every single contribution, the alternation between the discussion of general points and their illustration by special examples, the illumination of the same problem (e.g. orthogenesis or quantum evolution) from the various angles of different methods and different minds, all combine to make the volume a most valuable and stimulating contribution towards a fuller understanding of the forces, trends, rates and achievements of evolution. The excellent glossary is of particular value in a book of this nature, which appeals to workers in many different fields. C. AUERBACH

Principles of Human Genetics. By CURT STERN. Pp. 628. San Francisco: W. H. Freeman and Co. 1949. $7·50.

There is a section in a recent book by Darlington and Mather entitled 'The Special Uses of Man'. His one-egg twins, his blood, his tumours, and above all the gift of speech which betrays his mind, have engendered much fruitful research. He has also an unrivalled collection of inherited diseases and defects which lead to knowledge of his biochemical genetics, partial sex-linkage, and so on. In short he is not only an important subject for certain kinds of research, but now provides material for quite a comprehensive text on genetics for undergraduate students. Those, like medicals, who are not particularly concerned with historical development and have no intention of specializing, would be well suited by such a treatment of the subject. Dr Curt Stern has seized this opportunity. With characteristic clear thinking and a disinclination to go a whoring after his own or anyone else's inventions, he has provided an excellent text for the class of student he has in mind. In this case student means student, not a busy clinician or family adviser who wants a snappy answer to genetic problems he may encounter.

Dr Stern's book is not a compendium of human inheritance, but a successful attempt to teach the basic facts of heredity in terms of man's own variability—from baldness to the *Rh* story, from haemophilia to racial miscegenation. 'Genetic Counseling' is not ignored, but it is for the most part limited to clear-cut Mendelian situations affecting individual families. Counseling on the grand scale, that is by eugenists of the positive or negative persuasions, does not receive much encouragement. 'Human Engineering', like livestock improvement, has passed through a phase of over-confidence arising from too little knowledge into a much more cautious state. From fairest creatures we desire increase, but this is not just a matter of simple Mendelism. If these fairest creatures are to be mentally and physically desirable, there are problems of quantitative genetics ahead which may not be as difficult as the social and ethical problems, but are intractable enough. There is obviously much to come, but for readers who want an authoritative, clearly written conspectus of the present state of human genetics demanding no specialized knowledge, this is the book. H. P. DONALD